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Guide to Adaptive Immune Receptor Profiling Technologies

Overview of Cellecta's DriverMap™ Adaptive Immune Receptor (AIR) Profiling Assay

Cellecta's DriverMap™ AIR profiling assay (Fig.1) is a next-generation DriverMap targeted multiplex RT-PCR technology designed for bulk expression profiling in a single assay of **all functional** T-cell receptors (TCR) and B-cell receptors (BCR): TRA, TRB, TRD, TRG, IGH, IGK and IGL mRNA isoforms (Fig. 4, 5) excluding non-functional pseudogenes and ORFs (as defined by the IMGT database http://www.imgt.org/IMGTrepertoire/). To achieve high performance profiling of the CDR3 receptor variable region involved in recognition of foreign antigens, we experimentally validated and selected the 315 best performing forward primers designed to target the FR3 (variable) region, and 19 reverse TCR/BCR primers designed for the conservative (C) region of TCR and BCR mRNAs. Furthermore, the novel multiplex RT-PCR protocol significantly reduces the level of non-specific/off-target and primer-dimer amplification products (Fig. 2). The DriverMap AIR assay is designed with Unique Molecular Identifiers (UMIs), which facilitates the accurate quantification of the copy number of cDNA molecules used in amplification steps, and detection of low abundance clonotypes above background level. Using UMIs also allows us to identify and correct amplification biases and sequencing errors, which is especially important in detection of medium- to low-abundant clonotypes. The Cellecta DriverMap AIR assay is based on the use of a dual-index amplicon labeling strategy to minimize index-hopping NGS shortcomings, and the Illumina 300-n paired kit for CDR3 sequencing. NGS data analysis is supported by the leading MiXCR software package, available online [11] or in Illumina's BaseSpace. The MiXCR-VDJ immune repertoire software allows one to generate interactive reports displaying comprehensive somatic mutation profiling across the antigen-recognition CDR3 region, accurate clonotype quantitation, CDR3 length distribution, V(D)J segment usage, and isotype composition for BCRs.

The DriverMap AIR assay allows one to simultaneously profile all CDR3 TCR and BCR clonotypes together (or separately all TCRs and/or BCRs) in a single test-tube reaction, which is difficult to do with other conventional technologies (Fig.3). Comprehensive analysis of both TCR and BCR repertoire is a promising strategy for unbiased discovery of predictive and prognostic biomarkers considering that both arms of the adaptive immune system work synergistically for many disease or adaptive immunity stimulus conditions [13,18].
Furthermore, the AIR assay is a single test-tube assay which doesn't require any intermediate purification steps or additional specialized equipment. A s imple protocol with minimum number of intermediate steps allows one to generate 96 amplified CDR3 products ready for NGS in one day. The AIR assay is based on a highly validated primer set combined with very low off-target background. The DriverMap AIR protocol allows one to achieve highly comprehensive immune receptor repertoire coverage for both small samples (e.g. 1-1K T/B cells) or bulk profiling in large samples (e.g. whole blood or biopsy tissues).

The DriverMap AIR assay is available as a kit (24 or 96 samples) or as a custom service from Cellecta.

Selection Strategy for Adaptive Immune Receptor (AIR) Profiling

The main goals of Cellecta's AIR product line are to: 1) characterize the global AIR repertoire in normal and pathological conditions; 2) identify disease- or pathogen-specific TCR and BCR clonotypes (both CDR3 sequences and chain pairing); 3) characterize the phenotype of antigen-specific activated T- and B-cells; and 4) match CDR3 sequences with the HLA genotype (for TCRs). Currently, most AIR technologies available on the market are based on amplification of TCR/BCR sequences followed by NGS, and provide only partial information [1-7,18] with significant systematic biases between different technologies [8, 14]. In the following sections we will provide practical guidelines for selecting the best design strategy and for implementation of AIR experiments.

Choice of RNA vs gDNA template

Traditionally, both genomic DNA (gDNA) and RNA have been used for AIR profiling, each with advantages and drawbacks. Our preference is to use RNA as a first choice for immune receptor profiling, which has significant advantages over gDNA [1,2]:

1) ***Sensitivity***. The copy number of mRNA per cell is at least 10-100-fold more than gDNA. This provides a significant increase in sensitivity due to the higher number of starting RNA template copies isolated from biological samples [4]. Increased AIR sensitivity using RNA templates is an important advantage for reliable profiling of TCR/BCR clonotypes in small biological samples (e.g., sorted cells, tumor, FFPE, etc.).
2) ***Functionality***. Using RNA as a template limits amplification to only "functional", expressed receptor chains. AIR profiling from gDNA amplifies a significant number of additional sequences, including non-rearranged and non-functional (pseudogenes and ORFs) receptor genes. This significantly increases undesirable background in the NGS data especially in tissue samples with low content of immune cells.
3) ***Coverage***. BCR repertoire analysis using gDNA as a template doesn't allow one to identify the Ig isotype as V(D)J and C regions are separated from each other by an intron and cannot be amplified together in a multiplex PCR reaction.

The drawbacks of using RNA include the requirement for reasonable RNA template integrity (RIN > 5). Furthermore, accurate quantitative analysis of T/B cell number for each clonotype could be performed only by using calibration standards and gDNA as a template. Please note that precise quantitation of cell numbers for each CDR3 clonotype (based on receptor gene copy number) is not a very critical issue if a goal of your research is detection of "up-regulated"/amplified disease-associated clonotypes (similar to differential gene-expression analysis), e.g. in serial/time course AIR profiling experiment in blood samples.

Identification of disease-activated CDR3 clonotypes

A significant body of evidence demonstrates that activation of adaptive immunity induces significant up-regulation of both BCR and TCR transcription in antigen-specific clonotypes (e.g., up to 1,000-fold for plasma B cells [13]). As a result, the RNA-based immune receptor repertoire is usually dominated by a low number of abundant clonotypes and many of these abundant clonotypes could be antigen-induced or disease-specific. Up-regulation of TCR/BCR receptor gene expression in activated effector/memory T- and B-cells could be used to identify and differentiate antigen-induced CDR3 sequences from bystanders non-activated CDR3 sequences which are commonly present in tumor samples [20]. Furthermore, transcriptional up-regulation of TCR genes in large CD8+ effector memory clonotypes in peripheral blood samples was associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma [23]. AIR repertoire profiling of overlapping transcriptionally activated CDR3 clonotypes in tumor, blood and tumor-draining lymph nodes could be a very promising strategy to identify tumor-reactive T cells [24].

We believe that the best strategy to differentiate antigen-induced clonotypes from abundant "naïve" clonotypes which are commonly present in cancer tissue samples is to generate and normalize mRNA-based to DNA-based CDR3 profiling data, as illustrated in Fig.12. In order to facilitate these studies, we developed an additional set of reverse AIR primers complementary to the J region of all TCR and BCR genes (Fig.13) which could be used in combination with Fwd FR3-region specific primers to amplify CDR3 sequences from genomic DNA (genDNA). As a result, using the combination of Fwd FR3-region with Rev C-region or with Rev J-region allows the generation of mRNA-based and genDNA-based AIR profiling data, respectively.

As a practical guideline, we propose to perform AIR profiling from both RNA and genDNA isolated from the same biological sample (e.g. PBMC with high content of immune cells). In another approach, both mRNA-based and genDNA-based AIR profiling could be performed directly in T- and/or B-cells sorted from tissue or blood samples (see below). Furthermore, UMI-based AIR profiling generated by MiXCR software could be used to normalize mRNA-based to genDNA-based profiling data to reveal disease-associated CDR3 clonotypes.

Bulk vs Single-cell Analysis

Bulk analysis is a front-line, high-throughput experimental strategy for comprehensive and sensitive detection of disease or treatment-associated clonotypes. It can be performed using RNA isolated from either small or large samples of frozen or lysed blood, cell fractions or tissues. Additional advantages of bulk analysis are its use of simple protocols which can be easily scaled-up for analysis of dozens to hundreds of samples, the reasonable cost of sequencing, and the wide range of available user-friendly data analysis software tools. The main shortcomings of bulk analysis are its inability to provide information about pairing of receptor chains or the phenotypes (e.g., T-cell subtyping) specific for each clonotype. Although cell type deconvolution algorithms can potentially link cell subtypes with the most abundant clonotypes, the practical application of this strategy is currently rather limited [10]. A more promising strategy is immune receptor profiling in immune cell fractions (e.g. T/B naïve, memory, effector, exhausted) isolated by FACS or by antibody-magnetic beads. Combined AIR-phenotype analysis in sorted T-cell fractions was successfully used for discovery of novel immunotherapy biomarkers [13,18] but still missing chain-pairing information.

Single-cell AIR is a revolutionary approach which allows investigators to combine clonotype repertoire with paired-chain information and the phenotype (e.g., cell subtype) of cells which express specific TCR/BCR sequences and their expression of key T- and B-marker genes. Single-cell AIR assays can be performed in low throughput (96-384 cells) using sorted cells in 96-well plates (e.g., DriverMap AIR assay) or in medium throughput (1,000-5,000 cells) using microwell arrays (BD Rhapsody) or droplet microfluidics (10xGenomics) technologies [1-5,9]. Importantly, single-cell AIR assays are more complicated and significantly more expensive (for both reagents and sequencing) than bulk assays, and typically require use of live cells, which is not possible for many applications. Furthermore, single-cell AIR can only profile the most abundant clonotypes due to the low number of cells used in the assay. As a result, single-cell AIR assays may be best used for combining immune repertoire and cell phenotyping information for the most abundant clonotypes identified by bulk analysis or for discovery of unique clonotypes significantly enriched in pathological (cancer) tissue samples relative to control blood samples.

Clonotype Coverage and Detection Sensitivity

Comprehensive Clonotype Analysis. Based on deep NGS sequencing, normal individuals have an estimated, at least, 100-200 million different TCR and BCR clonotypes present in the circulation (peripheral blood) with approximately 1-10% of shared clonotypes between any two people [15-17]. As a result, comprehensive characterization of the full immune repertoire would require analysis of RNA samples isolated from at least 10 billion lymphocyte cells (e.g. leukapheresis blood samples) which is not practical for most applications. Moreover, comprehensive AIR profiling is more challenging than conventional RNA-seq because of the enormous complexity (at least 1000-fold more) of different clonotype sequences versus the number of protein-coding genes (approximately 20,000). Importantly, the majority of clonotypes are present at very low single-cell levels, while a small number of high-to-medium abundant clonotypes (only few thousands) are overrepresented in NGS profiling data (although they account for only 20-30% of total reads), as illustrated in Fig.4 for AIR TCR (beta chain CDR3) repertoire profiling in a normal whole blood and PBMC RNA sample.

The long-tailed clonotype distribution, with the majority of reads corresponding to low abundant clonotypes, results in significantly reduced efficiency of detecting medium abundant clonotypes which could be represented by only a few molecules in the RNA sample (Fig.4). From a practical point of view, this means that in most experimental settings (with a limited number of analyzed lymphocyte cells and limited amount of total RNA) we may reliably detect only the most abundant (high-medium) clonotypes and will always lose (fail to detect) a significant fraction of low abundance clonotypes which are "randomly" present in RNA samples at single mRNA molecule level. For biological samples with a small number of lymphocytes (e.g., tumor samples, immune cell fractions, FFPE, etc.) or for single-cell AIR assays, comprehensive detection of both high and medium abundant clonotypes represents a serious technical challenge and in most cases is not possible.

To understand reproducibility of AIR data, the experimental plan should ensure that the number of sequencing reads exceeds at least 10-fold the clonal diversity of analyzed samples. To achieve this goal, it is important to perform replicate amplification and sequencing of the AIR library generated from the same RNA sample [18]. In addition to technical/amplification replicates, in an ideal case it is desirable to repeat AIR profiling from at least two to three RNA samples independently purified from the same biological sample (e.g., blood), although in many cases it is not possible, e.g. in immune cells isolated from tissue/cancer samples [18].

For reproducible, comprehensive analysis it is important to use the technology which provides the highest efficiency in amplification of both high and low abundant clonotypes using the small amount of RNA (e.g. 10-100ng). Currently, multiplex PCR and 5'-RACE (SMART) are the two commonly used targeted CDR3 amplification technologies used in AIR assays [1-5]. Recent benchmarking studies of commercially available AIR assays demonstrated that 5'-RACE (SMART) technology for RNA templates and multiplex PCR for genDNA templates have the best sensitivity in comprehensive repertoire analysis [8,9]. To achieve even more reliable and comprehensive detection of immune repertoire in RNA samples, Cellecta has developed a novel ultra-sensitive, multiplex RT-PCR DriverMap AIR assay designed for both bulk and single-cell immune repertoire-phenotype analysis (see Fig.3).

DriverMap™ Bulk AIR Assay Performance

Starting with the same amount of total RNA (50 ng of PBMC RNA), the DriverMap AIR assay identifies three-fold more clonotypes than a conventional leading SMART-based immune profiling assay (Fig. 6). Both technologies identify a similar set of top abundant TCR clonotypes (Fig. 7) with a less reproducible detection of medium-low abundant clonotypes for both technologies (Fig. 8). Moreover, due to increased sensitivity, reproducibility in triplicate RNA samples is significantly higher for DriverMap than for the SMART technology (Fig. 9). Using less RNA (2-10 ng) decreases the number of reliably detected high-medium abundant clonotypes several fold (Fig. 10).

In additional studies, we compare AIR assay performance to a leading multiplex PCR protocol designed for TCR/BCR profiling from genomic DNA [8]. As a template for the AIR assay we used RNA isolated from multiple sets of sorted T- and B-cells from metastatic tumor samples. In parallel studies, the genomic DNA isolated from the same cell fractions were used for multiplex PCR TCR/BCR assay [20]. As illustrated in Fig.11, AIR protocol identifies 1.5-2-fold more clonotypes than genDNA-based multiplex PCR protocol.

Applications of AIR profiling assay

- **Deep Clonotype Profiling from Large Lymphocyte Cell Number.**

  The AIR assay could directly use total RNA (without globin, rRNA or mitochondrial RNA depletion) isolated from whole blood, buffy coat, PBMC, lymphoid tissues, immune cell fractions (e.g., isolated by

magnetic beads), bone marrow aspirate, etc., e.g. samples with high content of lymphocyte cells. Due to improved sensitivity, the DriverMap AIR assay allows one to reliably detect up to 3,000-5,000 TCR clonotypes (without significant drop-out rate of medium abundant clonotypes) starting from 50 ng of PBMC total RNA (triplicate samples) as illustrated in Fig. 5. Increasing the starting amount of total PBMC RNA (from 50ng to 250ng) increases the number of detected rare clonotypes but doesn't improve--and in many cases reduces--efficiency of detection of medium-high abundant clonotypes due to practical restrictions in sequencing depth and reduced efficiency of consensus CDR3 sequence alignment. Using less amount of PBMC RNA (e.g. 10 ng) reduces accuracy of profiling medium abundant clonotypes. As a practical guideline, these results demonstrate that for RNA samples purified from large numbers (more than 1-2 million) of lymphocytes, 50 ng of total RNA is an optimal amount for deep profiling of TCR/BCR clonotypes using the DriverMap AIR assay.

In order to simplify collecting and processing multiple (e.g. serial time course) blood samples, we demonstrated that AIR data could be easily generated from whole blood RNA samples (after purification of RNA from whole blood collection in Tempus or PAXgene test tubes) (Fig. 4). Using whole blood instead of commonly used PBMC as a starting material could significantly reduce batch effects due to differences in quality of PBMC preparation induced by variation in preparation, storage and transportation conditions [12]. Side-by-side comparisons between whole blood and PBMC also show significant correlation in the repertoire of detected high-medium abundant clonotypes (Fig.6-7). Based on these results we recommend using whole blood (stabilized in Tempus) rather than PBMC for deep AIR profiling in blood samples.

For reliable detection of clonotypes which are activated during infection, pathological processes or treatment conditions, it is critical to measure accuracy of AIR repertoire profiling. To calibrate the AIR profiling data to copy the number of CDR3-specific target mRNA molecules used in the assay, universal molecular indexes (UMI) are incorporated in reverse gene-specific primers (Fig.2). For a highly abundant class of approximately 500 CDR3 clonotypes (amplified from at least 10 mRNA molecules) the reproducibility of clonotype abundance measurement is high (Fig.7). Unfortunately, for the most represented class of medium abundant clonotypes (3,000-5,000 clonotypes amplified from at least 3 mRNA molecules) the measured abundance level has significant variation due to the low number of CDR3-specific mRNA molecules used in the assay (Fig. 5). Detection of the most represented low abundant class of CDR3 clonotypes (appr. 100,000) is not reproducible (as we discussed above) or quantitative as it "randomly" generated from single mRNA TCR/BCR molecules of very high complexity total RNA sample (comprising millions of different immune receptor mRNAs). Therefore, for comprehensive detection of activated high-medium abundant clonotypes, assessing its statistical significance and separating from the class of low abundant clonotypes, it is highly desirable to run each sample in technical triplicates. AIR profiling without triplicates is limited to analysis of only highly abundant clonotypes and could generate a significant number of false-positive, up-regulated, medium abundant clonotypes in multiple sample comparison studies. Furthermore, we recommend using approximately 5-10 million reads per sample (with approximately at least 20 reads per UMI) for deep AIR analysis in blood samples.

- **AIR profiling in tissue or fluid samples with Small Number of Lymphocyte Cells.**

  Due to the high sensitivity and low level of non-specific background products, in many cases AIR profiling could be performed directly in total RNA isolated from cancer biopsies, non-lymphoid tissue, biological fluid, FFPE samples, etc. (Fig.8). We recommend purifying RNA from at least 1mg of tissue

samples; smaller samples (less than 1 mg or FFPE samples) could be analyzed directly (see below) without RNA isolation. For bulk CDR3 profiling in tissue samples with small numbers (e.g., 100-10,000) of lymphocytes it is important to keep in mind that low abundant TCR/BCR clonotypes are derived from only one or at most a few cells. In order to capture the majority of receptor diversity (at least most abundant clonotypes) of such samples we recommend the use of as much RNA as possible (up to 1,000-2,000 ng of total RNA) in the AIR assay, using less RNA will reduce detection efficiency of rare clonotypes.

- **Whole Blood AIR Microsampling Technology.**

  To simplify the protocol for collection of blood samples for AIR analysis we developed a protocol and demonstrated that robust immune repertoire data for the top TCR/BCR clonotypes could be generated from just 30 ul of dried whole blood (approximately 50,000 -100,000 lymphocyte cells) collected using Neoteryx's microsampling technology (Fig. 9, 7, 8). AIR blood microsampling technology could generate a reproducible expression profile similar to bulk blood profiling of up to 200 of the most abundant clonotypes (with at least 20 UMI counts) (Fig.8). Quantitation of less abundant clonotypes (e.g. 500 with UMI 10 or more) is semi-quantitative and requires the use of biological triplicate microsamples.

  The revolutionary Neoteryx microsampling technology has significant advantages in comparison with blood collection using vacutainers. Namely, the microsamplers allow home-based sampling of precise volumes (e.g. 30 uL) of fingertip blood (lancet). The air-dried microsamplers could then be mailed, without a need for refrigeration, to a central laboratory for analysis. They have been used extensively for clinical research and monitoring of metabolites, drugs, and other analytes. Our data show their potential for similarly convenient AIR analysis. AIR whole blood microsampling technology is a very promising approach for collecting multiple samples for longitudinal assessment of the immune repertoire in a wide range of disease conditions [19].

- **Direct AIR profiling in isolated immune cell fractions and tissue microsamples**.

  Isolation of immune cells or immune cell fractions (e.g. by FACS of antibody-magnetic beads) from complex biological samples (e.g. tissues or blood cells) could significantly improve detection sensitivity of immune receptor molecules. Importantly, the AIR assay in most cases does not require RNA purification. Immune cell fraction(s) isolated from blood, tissue, fluid, or tumor samples could be directly used in the AIR assay (Fig.10). Based on our experience, direct use of isolated immune cells in the AIR assay significantly (5-10-fold) increases sensitivity of AIR detection which could be explained by significant loss of RNA in purification using conventional methods (e.g. Qiagen Micro RNA kits). Please note that micro-dissected (e.g. laser capture) tumor samples (with less than 1 mg) or small FFPE samples (e.g. from one slide) could be also directly used in AIR protocol without RNA purification. Considering the expected low complexity of TCR/BCR clonotypes in these low lymphocyte samples, we recommend using approximately 2 million reads per sample (or at least 20 reads per UMI).

- **Immunophenotyping of CDR3 clonotypes with T/B immunophenotyping assay**.

  In addition to AIR profiling, the sorted immune cell subfractions could be comprehensively phenotypically characterized by an additional targeted multiplex RT-PCR T/B Immunophenotyping assay based on DriverMap technology and using the same protocol as for the AIR assay (Fig.3). To develop the T/B immunophenotyping assay, we combined a list of more than 3,000 T and B-specific

cell subtyping and activation markers from more than 100 different public databases, commercial assays, and scientific publications. Furthermore, we designed several sets of redundant primers and validated the performance of these primers against a panel of 40 different immune cell types and activated PBMC and whole blood samples. As a result of these validation studies, we came to a list of approximately 500 highly expressed and robust T/B subtyping and activation marker genes which could be effectively used to confirm the quality of cell sorting, and to identify and deeply characterize different immune cell subtypes (Fig.11). In addition, we also offer a genome-wide expression profiling assay to measure the expression levels of 19,000 human protein-coding genes. If the goal of the research is unbiased discovery of novel immunity biomarkers, we recommend using the genome-wide expression profiling assay.
Combined AIR and T/B Immunophenotyping profiling in immune cell fractions (e.g. sorted using the most common cell typing surface markers) allow linking specific sets of CDR3 sequences with phenotypes of different immune subfractions (e.g. CD4. CD8, naïve, memory, cytotoxic, effector, etc.) as illustrated in Fig. 10.

Considering differences in complexity of CDR3 sequences and the number of genes in T/B immunophenotyping assay, we recommend splitting each sorted cell fraction in a 5:1 ratio (e.g. 25,000 and 5,000 cells), performing AIR and T/B immunophenotyping profiling in each fraction separately and to mix amplified samples at a 1:1 ration at the NGS step.

- **Single-Cell Custom V(D)J immune profiling service.**

In addition to bulk AIR profiling (kit and services), Cellecta offers a single-cell T/B immune profiling custom service using the 10x Genomics Chromium V(D)J assay. The Chromium Single Cell 5′ Library Construction Kit and V(D)J Enrichment Kit offer comprehensive, scalable solutions for measuring gene expression and immune repertoire information from the same cell. This protocol can be used to generate an enriched T-cell library and/or an enriched B-cell library, and/or a 5’ Gene Expression library from amplified cDNA from the same cells and then profile full-length (5’ UTR to constant region), paired T-cell receptor (TCR), or B-cell immunoglobulin (Ig) transcripts from 100-10,000 individual cells per sample. A pool of ~750,000 barcodes are sampled separately to index each cell’s transcriptome. It does so by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced and 10x Barcodes are used to associate individual reads back to the individual partitions. Detailed information about experimental design, sample preparation and downstream data analysis may be found at www.10xgenomics.com/support/single-cell-immune-profiling.

A single-cell V(D)J immune profiling and data analysis service is available for cryopreserved (in DMSO) lymphocyte cell samples (e.g. PBMC or sorted immune cells). For single-cell AIR experiments, it is desirable to use samples in which lymphocytes are enriched by centrifugation (PBMC), magnetic beads, or by FACS sorting. It is recommended to use low pressure sorting (if possible, or a nozzle $\geq$ 100 um). For all immune cell samples it is highly recommended to perform viability testing using live-dead staining to confirm that at least 70% of cells are alive. Upon receiving the cells, they will be processed using Chromium controller, a 5’-RNA expression profiling library will be constructed using RNAV(D)J protocol, sequenced and analyzed using Cell Ranger and Loupe V(D)J Browser software tools.

Additional DriverMap HLA Genotyping Assay:

The final goal of many AIR repertoire profiling studies is to identify target antigen epitope sequences recognized by the adaptive immune system. Several immune epitope databases were developed which accumulate published epitopes discovered in previous studies and algorithms for prediction of novel epitopes [6,7,21,22]. In order to match a set of activated by adaptive immunity CDR3 sequences identified in any study with published or predicted antigen-peptide-epitope sequences recognized by TCR/BCR, it is necessary to determine HLA allele/genotype for each experimental sample. The DriverMap HLA genotyping assay based on multiplex RT-PCR is designed for amplification and sequencing of exon2 and exon3 mRNA sequences of a complete set of HLA class I (A, B, C) and HLA class II (DMA, DMB, DOA, DOB, DPA1, DPA2, DPB1, DPB2, DQA1, DQA2, DQB1, DRA, DRB) genes in a single reaction. HLA Genotyping assay employs the same protocol as the DriverMap AIR assay and could be run from as small as 10ng of total RNA from whole blood, PBMC or tissue sample. HLA genotyping data analysis is supported by the MiXCR software package.

Early Access to Immune Repertoire Profiling (AIR)

Cellecta is offering an Early Access Program for DriverMap Immune Repertoire Profiling. If you have whole blood, PBMC, tissue, cell samples that you would like to have analyzed, please contact us at collaborations@cellecta.com for more information.

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Supplementary Figures

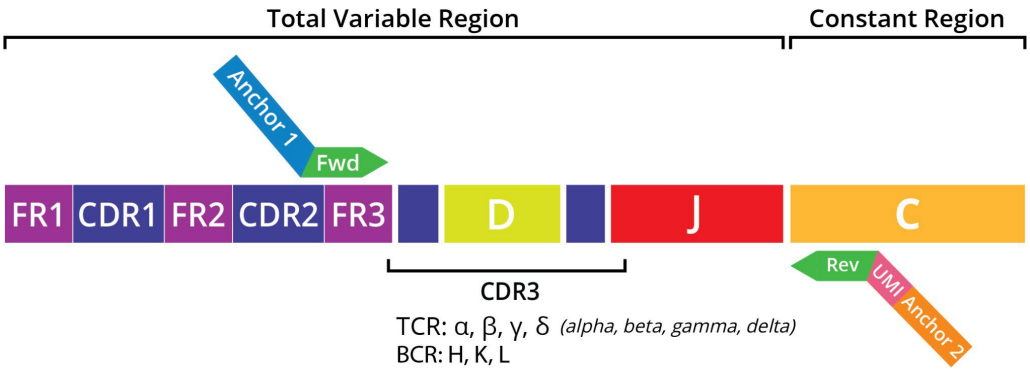

Fig. 1. mRNA structure for TCR (alpha, beta, gamma, delta) and BCR (heavy, kappa, lambda) chains and positions of forward and reverse PCR primers to amplify the CDR3 region in a multiplex RT-PCR reaction employed in the DriverMap AIR assay

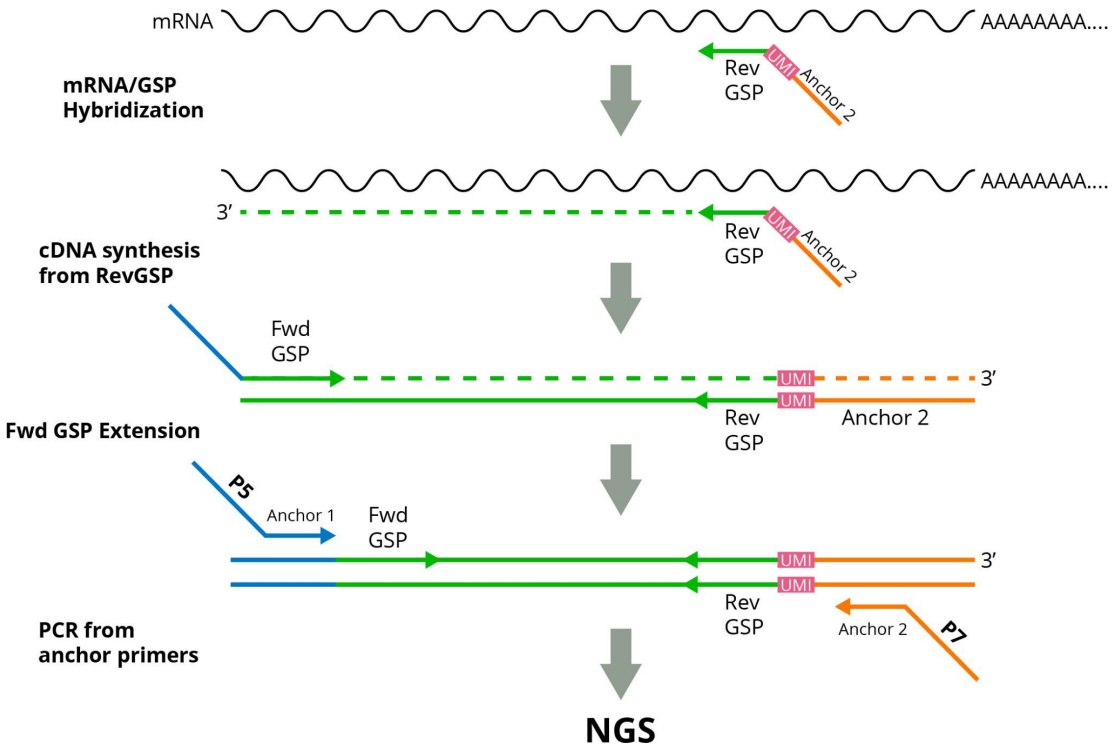

Fig. 2. Outline of DriverMap Multiplex RT-PCR Technology

Set of Reverse gene-specific primers (GSP) with incorporated UMI sequences targeting all TCR/BCR C-region isoforms, annealed and hybridized with RNA, extended with reverse transcriptase, followed by annealing and extension of a set of Forward GSPs using generated cDNA as a template. Anchored cDNAs are amplified by universal Anchor Primer 1 (AP1) and AP2 primers in the PCR step and analyzed by NGS for TCR/BCR CD3 clonotype sequences. For T/B immunophenotyping assay, reverse primer doesn't have UMI sequences.

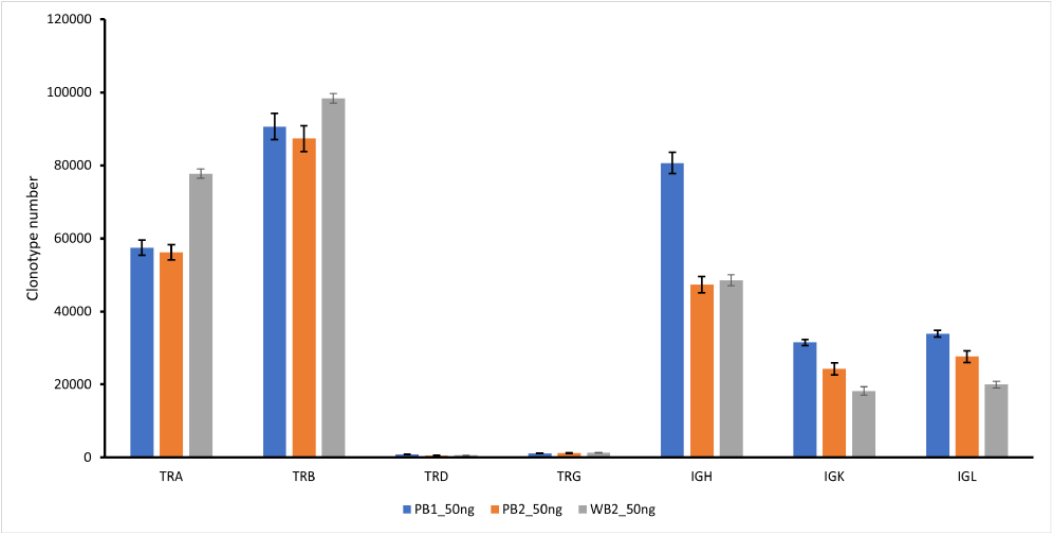

Fig. 3. Number of clonotypes for 7 TCR/BCR chains identified in 50 ng of normal PBMC or whole blood RNA. 10x10^6 reads per sample, triplicates.

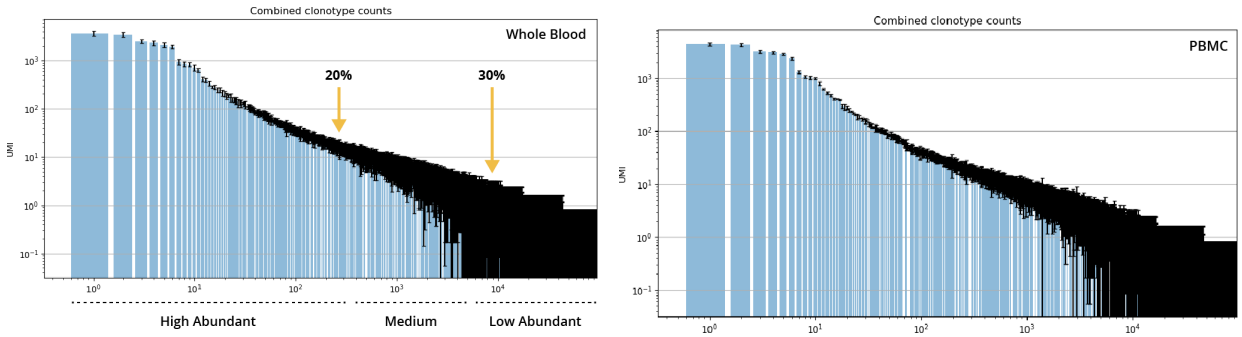

Fig. 4. Reproducibility of AIR TRB clonotype (high, medium, low) analysis for whole blood and PBMC RNAs (50 ng in triplicates). Black lines show variation in measured clonotype coverage. In the Y axis, 10^0 corresponds to a single TRB mRNA molecule in RNA PBMC sample. 20% and 30% are read numbers for top 500 and 10,000 TCR clonotypes.

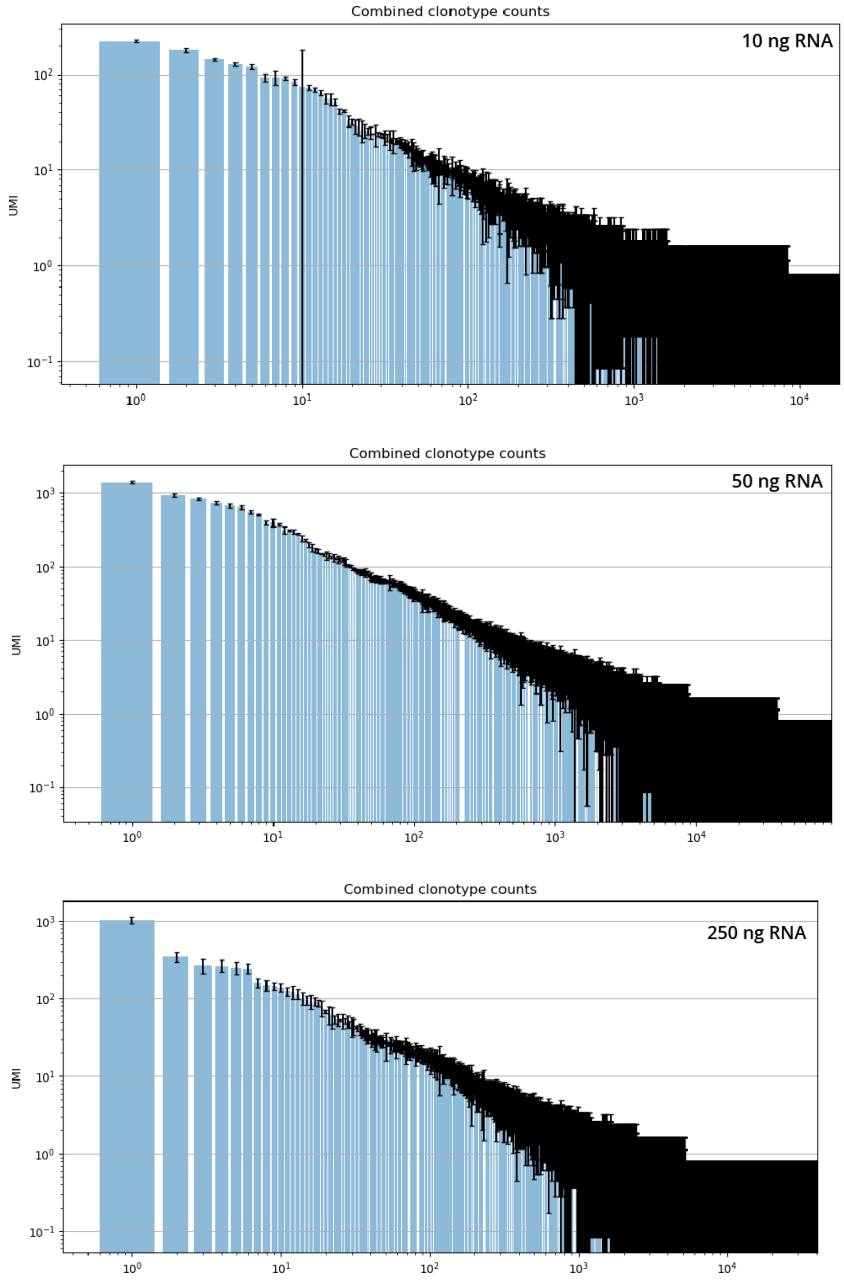

Fig.5. Reliability of detection of high-medium abundant clonotypes for 10 ng, 50 ng and 250 ng of PBMC RNA (in triplicates).

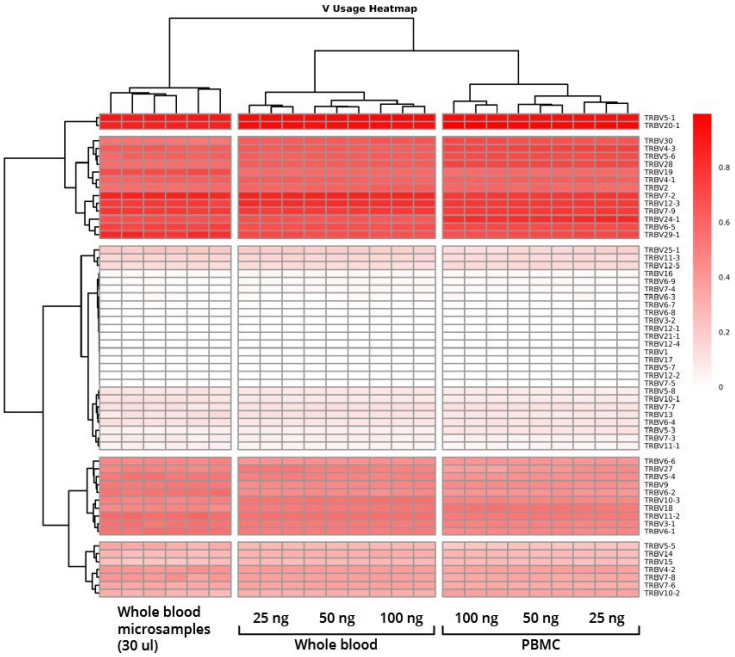

Fig. 6. Similar TRB V gene usage for whole blood, whole blood microsamples (30ul dried blood) and PBMC samples in the range of 25ng -100ng of total RNA. Median log10 UMI counts of 3 replicates

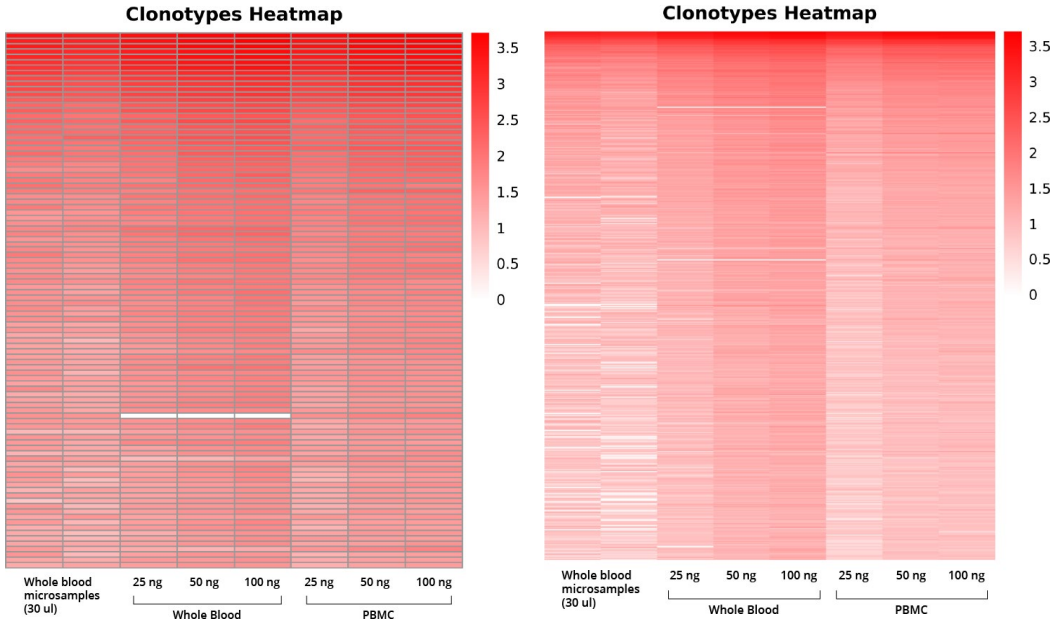

Fig. 7. Reproducibility in TRB repertoire profiling for top 100 and top 500 clonotypes for whole blood, whole blood microsamples (30ul dried blood) and PBMC samples in the range of 25ng -100ng of total RNA. Median log10 UMI counts of 3 replicates.

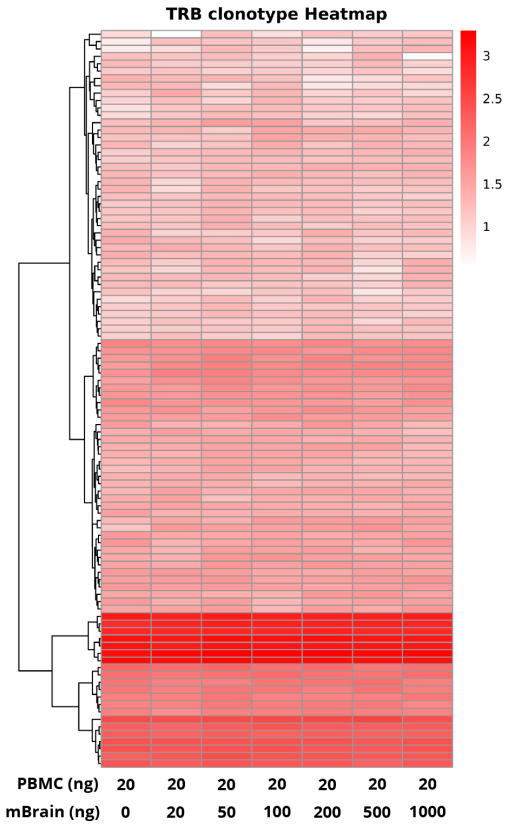

Fig. 8. Reproducibility in top 100 TRB clonotype profiling in 20 ng of PBMC RNA mixed with up to using 50-fold excess from mouse brain total RNA

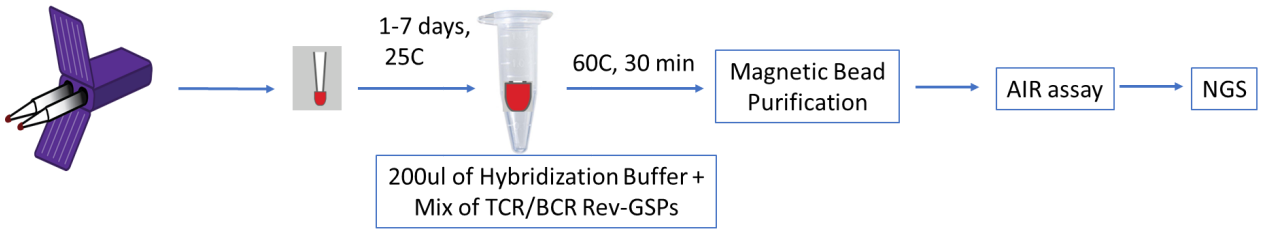

Fig. 9. Processing outline of dried blood samples using Neoteryx Microsampling technology.

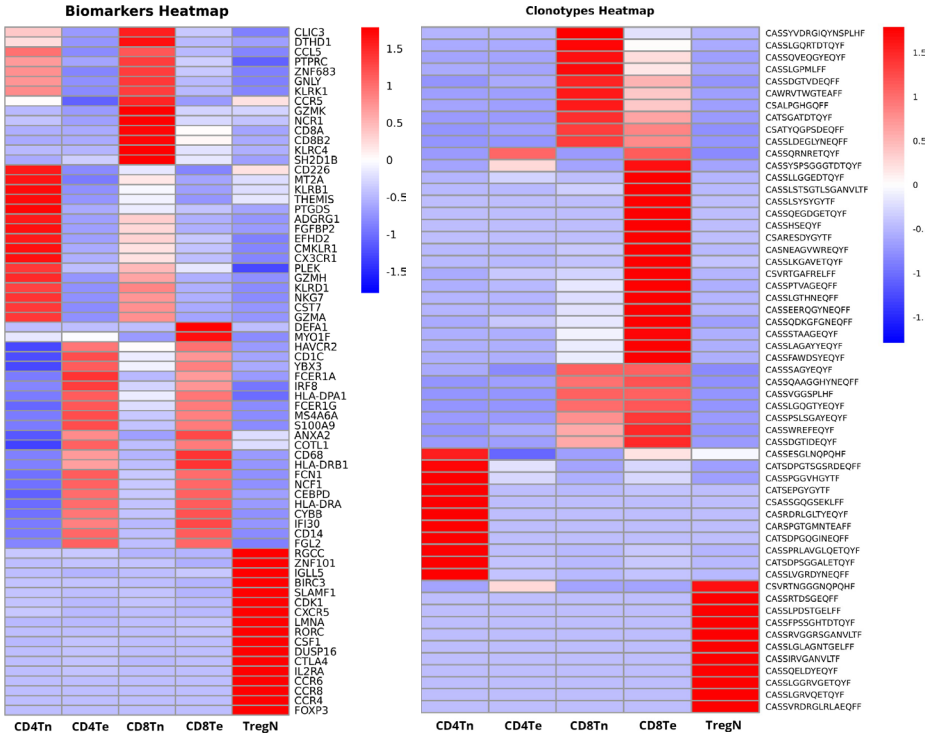

Fig. 10.

(A) – direct deep phenotypic characterization of CD4 naive, CD4 effector, CD8 naive, CD8 effector and Treg cells (25,000)
sorted from normal PBMC sample with T/B Immunophenotyping Assay.

(B) - Direct AIR profiling of TRB clonotypes in 25,000 of FACS sorted CD4 naive, CD4 effector, CD8 naïve, CD8 effector and T reg.

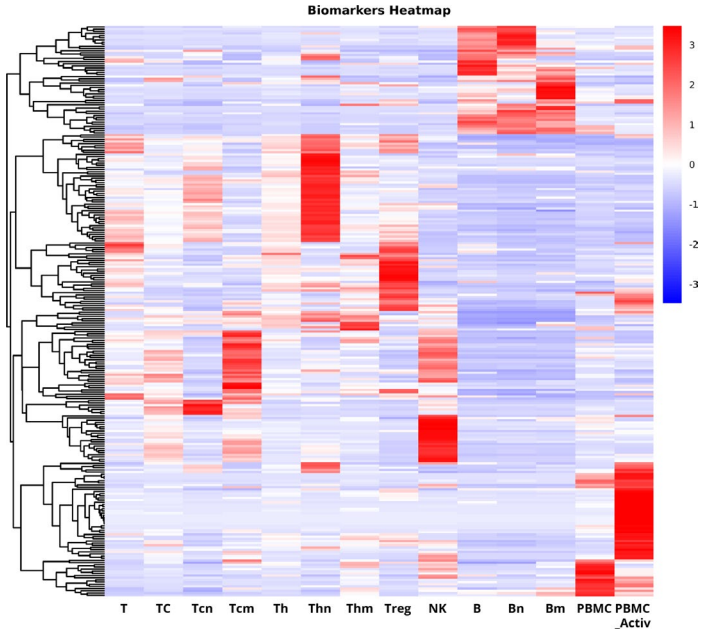

Fig. 11. Characterization of different T and B cell types (T, CD8, CD8 naive, CD8 memory, CD4, CD4 naive, CD4 memory, Treg, NK, B, B naive, B memory) using T/B Immunophenotyping assay.

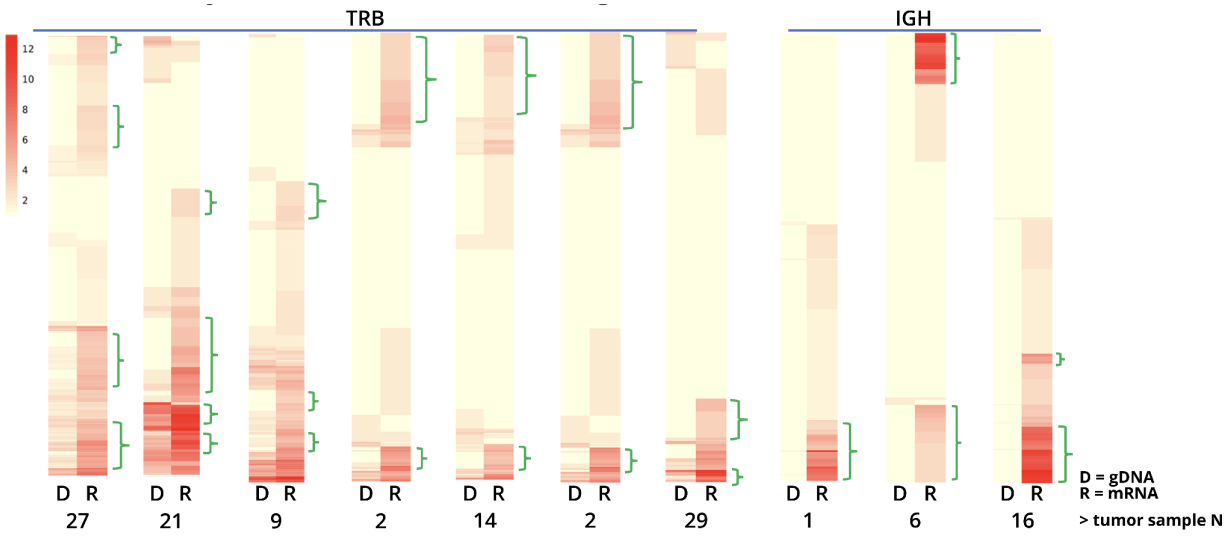

Fig. 12. Comparison of AIR mRNA-based to DNA-based profiling data allows to identify 5-10% of metastatic tumor-activated immune receptor clones. Tumor-activated CDR3 clonotypes (marked by brackets) characterized up to 1000-fold (B cells) and to 50-fold (T cells) transcriptional activation of immune receptor genes. (See Ref.20 for details)