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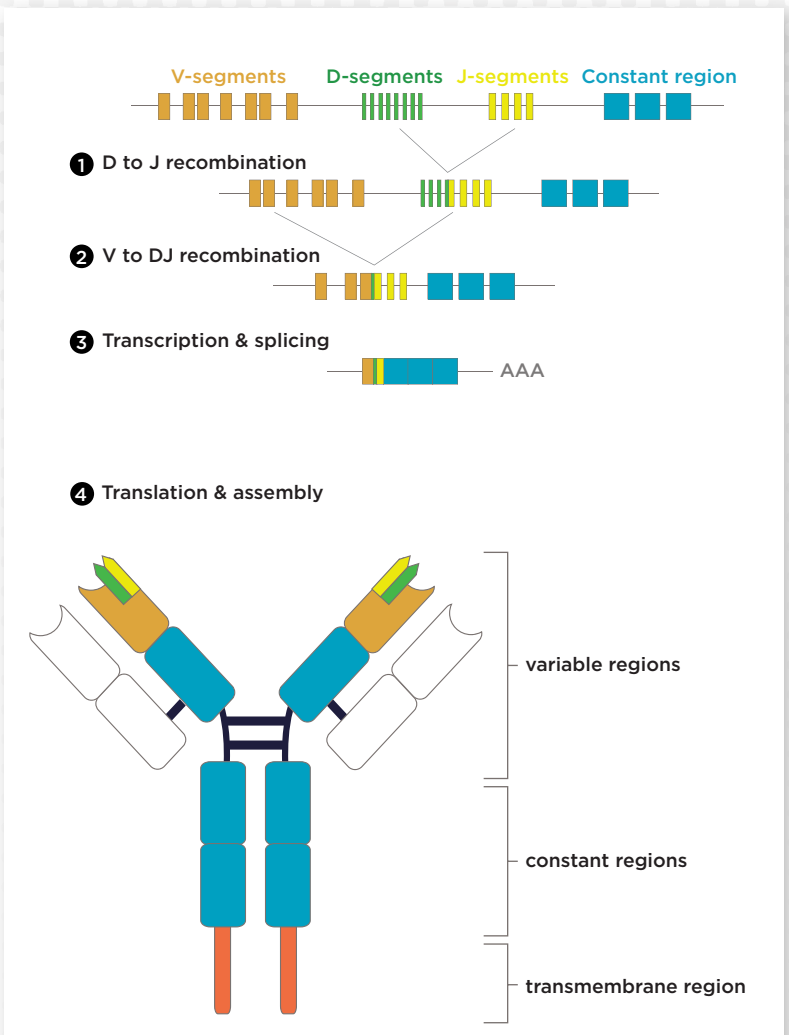
Revealing Immune Responses with Adaptive Immune Receptor Repertoire Profiling

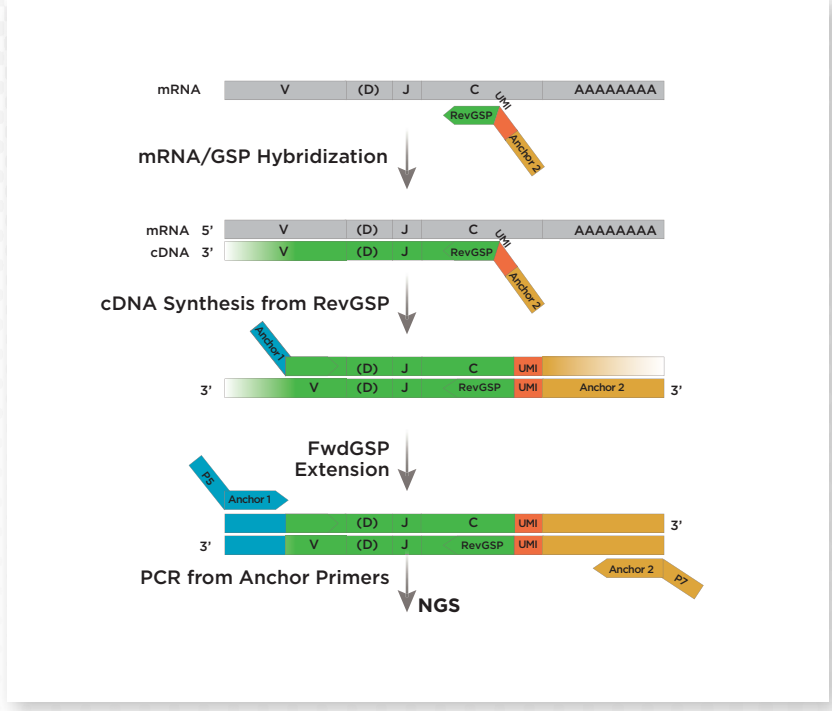
BACKGROUND: Receptor Diversity

T cell and B cell receptors (TCRs and BCRs) are key drivers of adaptive immunity. TCR and BCR coding sequences are arranged via a process known as V(D)J recombination. Within a given cell, variable (V), diversity (D), and joining (J) segments are randomly selected from many variants and combined to generate the full-length receptor. Scientists estimate that there are approximately 200 million T and B cell clones with distinct receptor sequence combinations present in human blood.



TECHNOLOGY: What Is the DriverMap™ Adaptive Immune Receptor (AIR) Repertoire Profiling Assay?

The DriverMap human and mouse AIR-RNA profiling assay uses targeted multiplex RT-PCR to profile all functional TCR and BCR isoforms (TRA, TRB, TRG, TRD, IGH, IGK, and IGL chains) in a single experiment while excluding non-functional pseudogenes and open reading frames. A single-tube multiplex RT-PCR followed by next-generation sequencing (NGS) enables robust rapid profiling of human or mouse RNA, DNA, or both. The simultaneous profiling of DNA and RNA enables the identification of antigen-activated clonotypes to provide insight into the immune response.



The Power of Immunosequencing

Immunosequencing amplifies rearranged CDR sequences, and when combined with high-throughput sequencing technology, has the power to enumerate and quantify thousands of TCR or BCR clonotype sequences simultaneously. Scientists can use this information to characterize the abundance and distribution of lymphocytes, as well as to track clonal migration over time during situations such as disease progression or immunologic responses.[2]

How Does the DriverMap AIR Profiling Assay Work?

1. Isolate total RNA or genomic DNA from any type of immune sample such as whole blood, PBMCs, cancer biopsies, tissue samples, FFPE, or dried blood microsamples.
2. Amplify AIR clonotypes either with the multiplex PCR-targeting CDR3 variable and conservative regions of the T- and B-cell receptors, or with the full-length assay that captures CDR1, CDR2, and CDR3 regions.
3. Use NGS with to profile clonotypes present in each sample. UMI-labeled primers enable quantitative analysis of clonotype representation.

To Start With DNA or RNA?

AIR repertoire profiling can be done with both genomic DNA (gDNA) and RNA, and each has its own pros and cons.

	RNA	gDNA
Sensitivity	10-100 fold higher than DNA	Relatively low
Functionality	Only amplifies functional/expressed genes	Amplifies many non-rearranged and non-functional sequences
Coverage	Can identify Ig isotype	Cannot identify Ig isotype
Quantitation	Cannot assess T/B cell counts per clonotype	Can assess T/B cell counts per clonotype

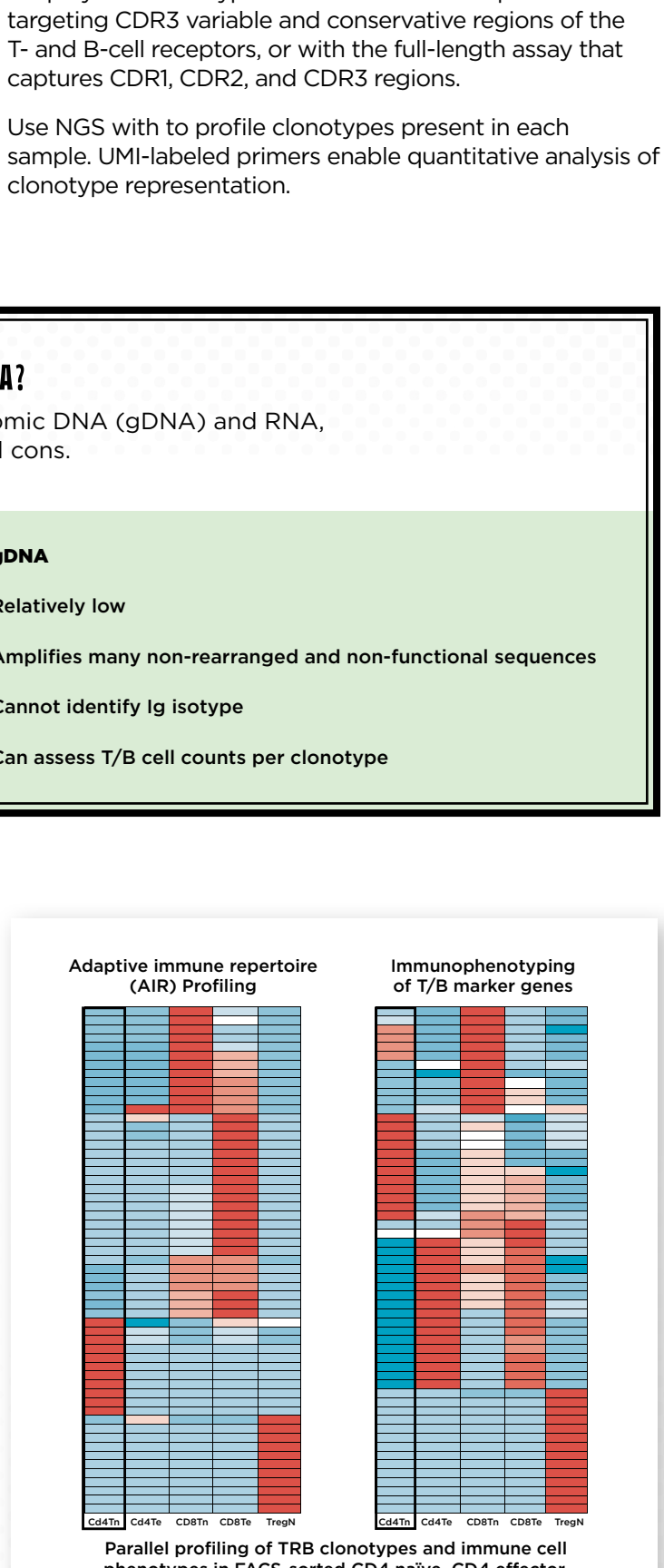
RESULTS:

The DriverMap AIR assay facilitates simultaneous profiling of all CDR3 or full-length variable region TCR (TRA, TRB, TRD, TRG) and BCR (IGH, IGK, IGL) clonotypes in a single test-tube reaction. The comprehensive analysis of both TCR and BCR repertoires helps researchers better understand how both arms of the adaptive immune system work synergistically.

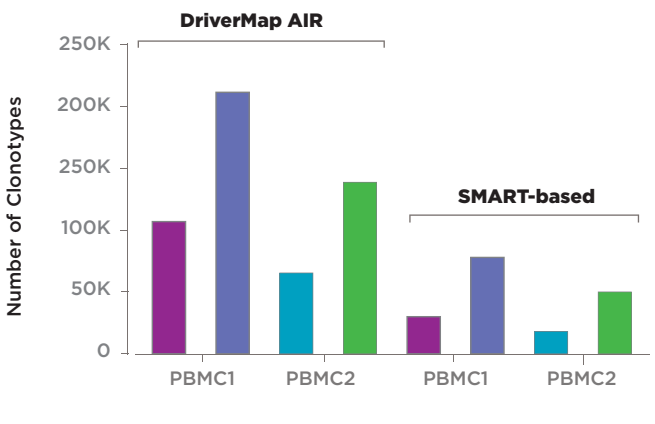
Running both AIR-RNA and AIR-DNA assays on the same sample allows for the identification of antigen-activated clonotypes by showing transcriptional activation of TCR and BCR genes in immune responses.

DriverMap AIR assay repertoire profiling is sensitive and reproducible. The assay reproducibly identifies and quantitates the most abundant 100-500 clonotypes (with specific mRNA copy number exceeding 10) present in RNA samples. It also detects 3,000-5,000 medium-abundancy clonotypes reproducibly if replicates are used. Finally, the assay amplifies 50,000-500,000 low-abundancy clonotypes which are present at single-copy mRNA levels.

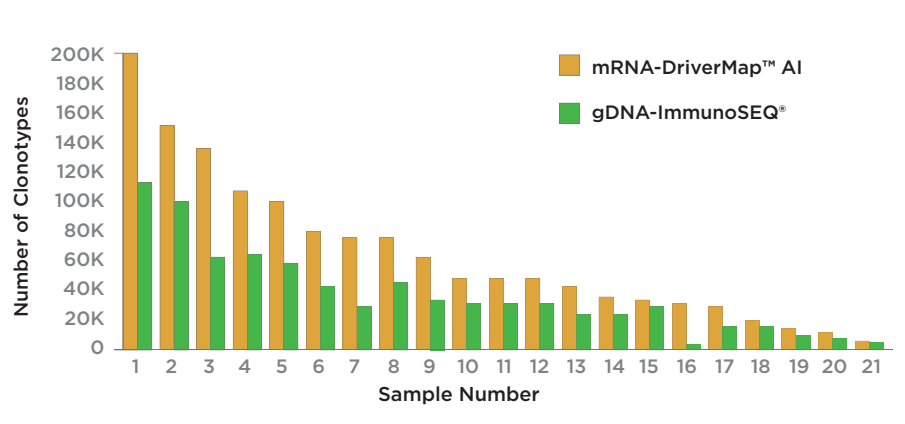
DriverMap AIR profiling and immunophenotyping assays complement each other. The direct parallel profiling of AIR clonotypes and targeted expression levels of 500 T/B cell-typing markers in sorted T cell fractions facilitates TCR repertoire characterization in specific immune cell subsets, including CD8 naïve, CD8 effector, CD4 naïve, CD4 effector, and regulatory T cell fractions, providing greater insight into immune samples.



Parallel profiling of TRB clonotypes and immune cell phenotypes in FACS-sorted CD4 naïve, CD4 effector, CD8 naïve, CD8 effector, and regulatory T cell fractions.



The DriverMap AIR assay identifies 3x more clonotypes than a conventional SMART®-based 5'-switch oligo TCR assay.



The DriverMap AIR assay detects 1.5-2x more clonotypes than conventional gDNA-based assays.

Bulk analysis or single-cell sequencing for AIR repertoire assays?

The bulk AIR assay is the best strategy for cost-effective, quantitative immune receptor repertoire analysis of a large number of biological samples with the goal of identifying antigen-activated clonotypes or disease/treatment-associated TCR/BCR biomarkers. Single-cell analysis is the most informative approach, but also the most costly strategy for the phenotypic characterization of cell encoding TCR/BCR clonotypes and receptor chain pairings identified through AIR bulk analysis.

	Bulk analysis	T/B cell fractions	Single-cell analysis
Throughput	High	Medium	Lower
Sample	Cells (intact or lysed), tissues, fluids	FACS-sorted cellular fraction or single cells	Intact live cells
Complexity and Cost	Easy, inexpensive	Modern, less expensive	Harder, more expensive
Scaling	Simpler	Less complex	More complex
Data Depth	Cannot provide information on receptor chain pairings; cannot link phenotypes with clonotypes	Provides information on receptor chain pairing for single-cell sorting; links cell phenotypes with clonotypes	Can link clonotype repertoire with paired-chain information and cell phenotype

Immune receptor repertoire profiling is an important analytic tool for disease research in many areas, including cancer,[2] cell and organ transplantation,[3] autoimmunity,[4] and infectious pathologies such as COVID-19.[5] The DriverMap AIR repertoire profiling assay offers researchers the ability to characterize their immune cell samples in a comprehensive and sensitive manner. The assays are available as ready-to-use kits or as a service with a rapid one-month turnaround.

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Helpful Resources

Comprehensive Adaptive Immune Receptor Profiling for All Immune Cell Types — flyer
Get an overview of the DriverMap AIR Profiling Assay for immune repertoire profiling starting from RNA or DNA

DriverMap AIR Repertoire (AIRR) Profiling Tech Guide
Learn all about the AIRR profiling techniques, strategies for designing experiments and key applications

T-cell and B-cell receptor repertoire profiling for biomarker discovery — scientific poster
View an outline of the DriverMap AIR workflow and a rheumatoid arthritis case study

Synthetic Spike-in Controls for Immune Repertoire Profiling — flyer
Ensure accurate and reproducible immune repertoire profiling results with this set of RNA controls

Imunophenotyping of T-Cell and B-cell Receptor Clonotypes for Biomarker Discovery — video (18:40)

For more information and to access the links to these resources, visit **Cellecta.net/ts-air-resources**

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Cellecta Inc. is a trusted provider of genomic products and services for drug target and biomarker discovery. Since 2006, we have collaborated with the world's leading pharma, biotech, government, and academic institutions. We apply our extensive expertise in viral vector production, functional screening, custom cell engineering and multiplex RT-qPCR to provide a variety of products and services, including:

- CRISPR and RNAi pooled libraries and loss/gain-of-function screening services to identify genetic pathways responsible for phenotypes and biological responses
- Cell barcode libraries and constructs for cell tracking and analysis of clonal variations within cell populations
- Transcriptome profiling, TCR /BCR profiling and digital spatial profiling for biomarker discovery
- Custom cell engineering projects for cell assay development, and more

From our headquarters in Mountain View, California, we work with researchers worldwide to power their discoveries. **Learn how to put our expertise to work for you at Cellecta.com**

References:

1. S. Teraguchi et al., "Methods for sequence and structural analysis of B and T cell receptor repertoires," *Comput Struct Biotechnol J*, 18:2000-11, 2020.
2. I. Kirsch et al., "T-cell receptor profiling in cancer," *Mol Oncol*, 9(10):2063-70, 2015.
3. A. Minervina et al., "T-cell receptor and B-cell receptor repertoire profiling in adaptive immunity," *Transpl Int*, 32(11):1111-23, 2019.
4. H. Katoh et al., "Immune repertoire profiling for disease pathobiology," *Pathol Int*, 73(1):1-11, 2023.
5. P.C. Taylor et al., "Neutralizing monoclonal antibodies for treatment of COVID-19," *Nat Rev Immunol*, 21:382-93, 2021.

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Immune receptor repertoire profiling. Your new superpower.

Introducing the **DriverMap™ Adaptive Immune Receptor (AIR) Profiling Assay**

Start with total RNA or DNA

- Comprehensive profiling of all 7 TCR/BCR isoforms
- Accurate detection of functional isoforms only, not pseudogenes or ORFs
- Robust results from blood, tissue, FFPE or any immune sample
- No specialized instrument required

Now available as a kit or as a service.
Learn more at cellecta.com/drivermap-air

Who we are

Cellecta Inc., a trusted provider of genomic products and services, has successfully collaborated with the world's leading pharma, biotech, government, and academic institutions since 2006. Our recognized expertise in viral vector production, functional screening, cell engineering and multiplex qRT-PCR has given rise to a portfolio of offerings useful for loss-of-function and gain-of-function phenotypic screening, cell barcoding, targeted RNA-Seq and adaptive immune receptor profiling, and more.

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