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Single-cell TCRα/β and TCRγ/δ immune receptor profiling and immunophenotyping using a 96-well plate sorted-cell approach

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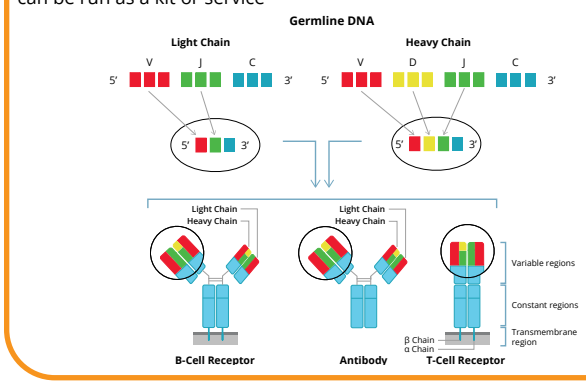
Abstract

Single-cell immune receptor profiling is a revolutionary approach that allows investigators to combine clonotype repertoire identification with paired-chain information and the phenotype of cells (e.g., cell subtype). Single-cell immune receptor profiling can be performed using a medium-throughput approach (1,000-5,000 cells) using microwell arrays or droplet microfluidics technologies. However, these assays are more complicated to run and require expensive reagents and limited sequencing throughput when compared to bulk immune receptor profiling methods. Here, we describe a low-throughput, single-cell immune profiling strategy using sorted cells in 96-well plates. The plates are pre-aliquoted with T-cell receptor (TCR) α/β and TCR γ/δ primers along with 30 crucial T-cell markers. We perform multiplex RT-PCR amplification and sequencing of the CDR3 regions. The resulting data provides the abundant clonotype sequences along with the chain pairing information for these TCR α/β and γ/δ chains, along with the T-cell subtype information using gene-expression profiles. By analyzing the TCR gene rearrangement at the single-cell level, researchers can better understand T-cell development, proliferation, and clonality, which are crucial for studying diseases such as cancer, immunodeficiency, and autoimmunity. Furthermore, single-cell TCR sequencing provides a user-friendly tool for the development of potential T-cell-based cancer immunotherapies. The technology's cost-effectiveness and ability to analyze clonotypes and immunophenotypes of cells in a single assay make it a valuable tool for detection and characterization of antigen-activated TCRs.

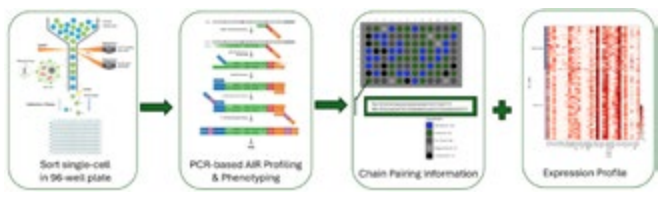
Introduction

- VDJ Mechanism of genetic recombination in T- and B-cells generates diverse repertoires of TCR, BCR and antibodies (secreted form of BCR)
- Variable part (CDR3) of TCR and BCR: recognize foreign antigens presented by MHC or recognized directly
- Hundred millions of different T- and B-cells with unique TCRs and BCRs: define differences in our immune responses
- Sequencing these diverse repertoire is called adaptive immune receptor (AIR) profiling or sequencing
- Single-cell AIR profiling allows to obtain **chain-pairing information** and **gene expression profile** of each cell-type
- However, single-cell assays are complicated to run and require expensive reagents and have limited sequencing throughput with the standard single-cell platforms
- Cellecta offers a 96-well plate based sc-AIR sequencing platform, which can be run as a kit or service



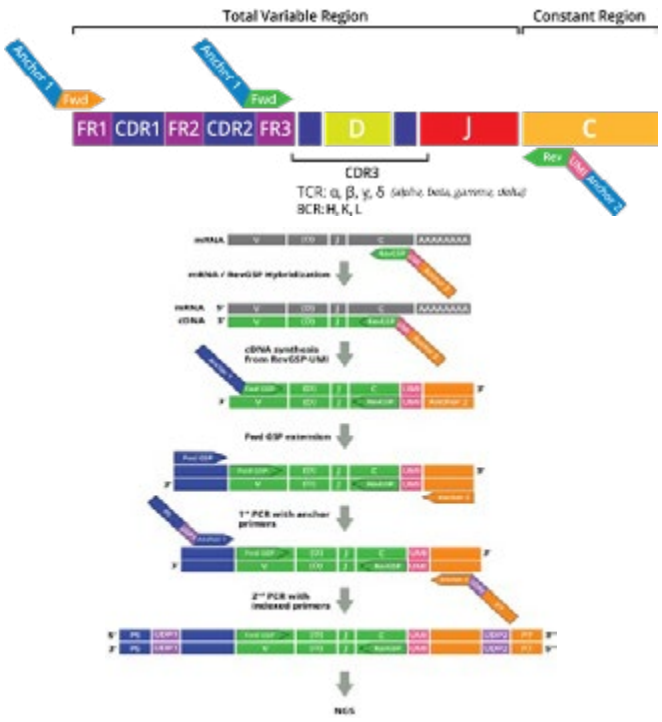
Method

- Single-cell FACS sorting in a 96-well plate format
- DriverMap™ sc-AIR profiling assay with RT-PCR based multiplex reaction, contains TCR α/β and γ/δ primer pairs with 30 crucial T-cell markers
- Primers contain UMI and well-specific barcodes with P5 and P7 adapters that can be sequenced on NextSeq 500/2000
- Results show sequences and chain-pairing information for TCRs along with phenotype of 30 Top T-cell markers for each sorted cell



Molecular Workflow

- RT-PCR based multiplex assay for TCR α/β an γ/δ primer pairs with 30 crucial T-cell markers.
- Improved coverage of CDR3 or CDR1-CDR2-CDR3 (full-length) regions with highly validated, redundant V primer sets.
- Unbiased amplification with universal anchor primers.
- No primer dimers and off-target products.
- Quantitative clonotype analysis with UMI and AIR RNA calibration standards.



Method (cont.)

Bioinformatic Pipeline

- Immune receptor profiling data is analyzed with the MiXCR software pipeline.
- T-cell marker gene expression profiling data is analysed with the Salmon/Alevin aligner.

Single-Cell AIR Validation

Plate Uniformity Test

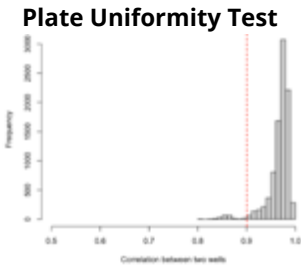


Fig 1: Conducting plate uniformity test shows >90% of correlation between 'normal' wells with a low SD.

Contamination Test

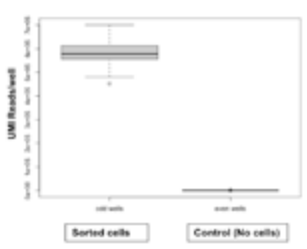
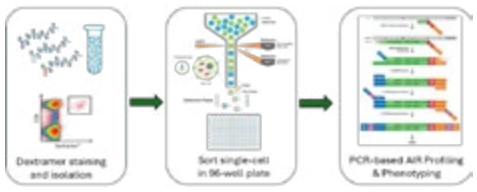


Fig 2: Cross-contamination test between odd wells (with sorted cells) and control even wells (without sorted cells). Low SD in odd wells with sorted cells shows the robustness of single-cell assay.

Case Study I: Detection of CMV-positive TCR Clone

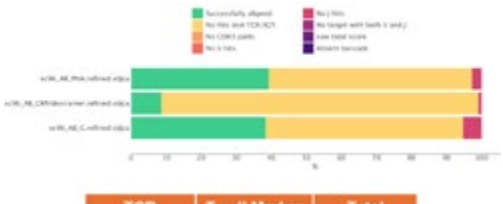
- Proof-of-principle experiment was conducted in CMV-positive PBMC stained single-cells with CMV-specific MHC-peptide Dextramer Reagent (Immudex) sorted in 96-well plate.
- As a control, we sorted non-stained single-cells (negative control) and PHA-activated single-cells (positive control) in a 96-well plate format.
- Single-cell AIR and T-cell marker amplification and sequencing was performed.



Results

Salmon Alignment

sample_name	num_processed	num_mapped	% mapped
sc96_AB_Control	16648353	9214806	55.34
sc96_AB_CMVdextramer	23046111	20787266	90.20
sc96_AB_PHA	16060064	9217344	57.40



TCR Clonotypes	T cell Marker Sequences	Total Alignment
40%	57%	97%
8%	90%	98%
40%	55%	95%

Fig 3: Read Alignment for T-cell marker gene expression (Salmon/Alevin) and TCR RNA expression (MiXCR) show a high total alignment percentage for all three samples.

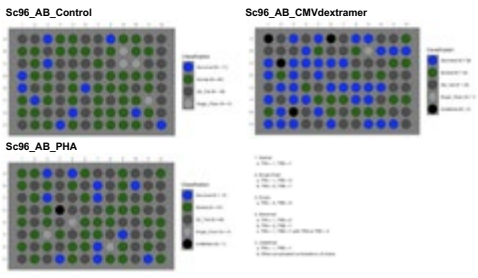


Fig 4: Classification of wells show the number of 'Normal' (green) wells that contain single chain information for TCR α/β clonotype pair. CMV Dextramer plate had a single activated clone in 19 wells.

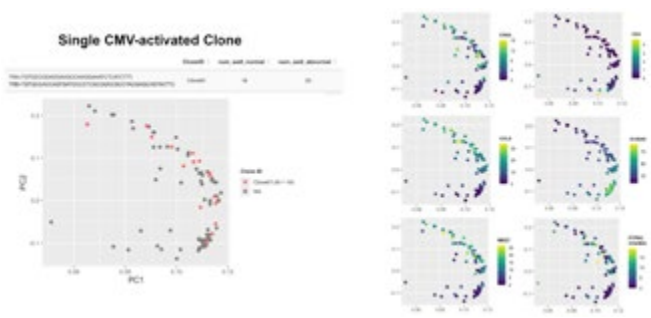
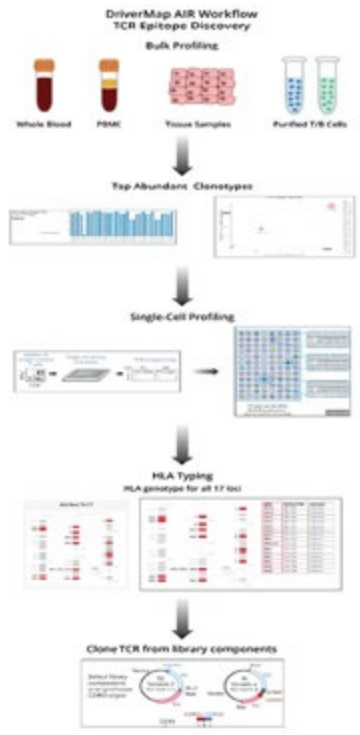


Fig 5: The single CMV specific dextramer clone pair (left) represents CD8+ cytotoxic cells and activated memory cell phenotype with high expression of effector genes NKG7, CCL5, GZMA, PRF1, IFNG etc (right).

Case Study II: T-cell Leukemia (Patient Sample)

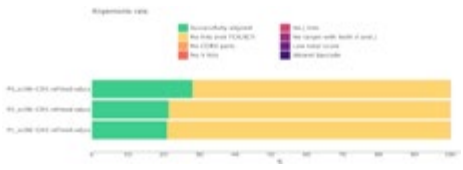


- Patient samples with T-cell large granular lymphocytic leukemias (T-LGL) at three different timepoints (3 plates) were analyzed using Cellecta's single-cell AIR-TCR-Mark30 profiling platform.
- Single-cell AIR profiling revealed most abundant TCR clonotype chain-pairs in a T-LGLL patient with severe neutropenia.
- Further analysis of the CDR3 region (VDJdb server) identified putative antigens recognized by the expanded clonotypes.
- The most abundant full-length receptor clonotypes were cloned and expressed in Jurkat cell lines and screened against candidate antigens to devise a model for the onset and development of T-LGL.

Results

Salmon Alignment

sample_name	num_processed	num_mapped	% mapped
P1_sc96-CD3	34191303	26734084	78.19
P2_sc96-CD3	31755140	24712502	77.82
P3_sc96-CD3	30210827	21451278	71.01



TCR Clonotypes	T cell Marker Sequences	Total Alignment
28%	71%	99%
21%	77%	98%
21%	78%	99%

Fig 6: Read Alignment for T cell marker gene expression (Salmon/Alevin) and TCR RNA expression (MiXCR) show a high total alignment percentage for all three plates.

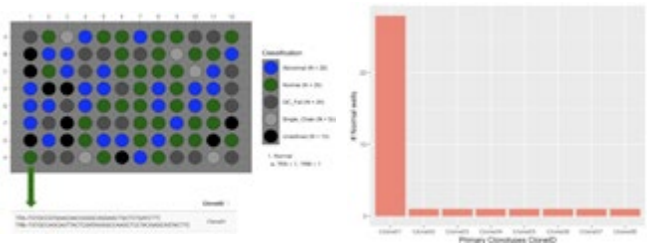


Fig 7: Most abundant single TCR α/β clonotype pair was identified in 35 wells (Normal) with single TCR α and TCR β chain across all three plates.

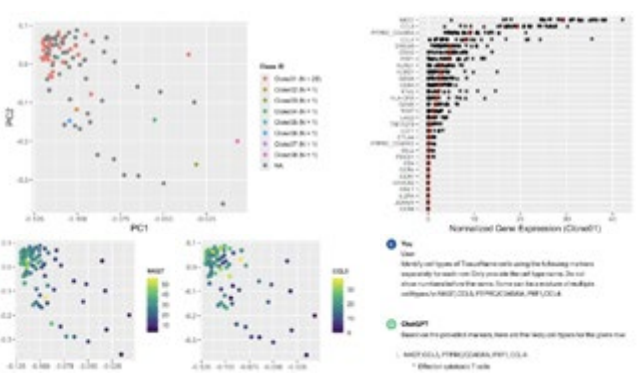


Fig 8: Gene expression profiling analysis revealed top cytotoxic effector markers such as NKG7 and CCL5 which are highly present in the most abundant clone. The most abundant clone present an effector memory cytotoxic T-cell phenotype.

Summary

- **Sensitive single-cell detection →**

Cellecta has optimized the multiplex RT-PCR protocol to provide sensitivity high enough to detect and profile T-cell receptor transcripts from single T-cells sorted using a flow cytometer. Low cross over between wells and UMI-based uniform clonotype detection allows accurate identification of sorted T-cells eg: antigen-activated T-cells labelled with specific MHC-peptide antigen.

- **Chain pairs & Immunophenotype →**

First of its kind 96-well plate-based single-cell AIR profiling assay that combines TCR chain sequencing with paired-chain information and the phenotype of cells (e.g., cell subtype) in a single multiplex assay without the need for any specialized instrument such as microwell arrays or droplet microfluidics-based single-cell technologies.

- **Cost-effective, efficient strategy →**

The 96-well plate-based multiplex technology allows it to be cost-effective to do single-cell profiling compared to any other service provider on the market which requires specialized equipment and expensive reagents to run a single-sample. Moreover, Cellecta's flexible pricing structure for the 96-well plate allows the researcher to scale the number of samples without scaling the profiling costs.