scientifix

PO Box 662 South Yarra
VIC 3141 Australia
www.scientifix.com.au

Free call: 1800 007 900
T: +61(0)3 8540 5900
F: +61(0)3 9543 7827
E: info@scientifix.com.au

High-throughput TCR profiling with 5'-end differential analysis of single cells

- High-throughput, high sensitivity clonotype and immune-cell-type identification from >1,000 cells in a single experiment
- Use of negative and positive controls in the experiment provides more confidence in data
- Simple GUI-based scTCR analyzer and mappa bioinformatics tools to speed up your data analysis

Introduction

The ability to profile T-cell receptor (TCR) expression at the single-cell level allows researchers to understand how particular alpha-beta (αβ) chain pairings contribute to the antigen specificity of individual TCRs. High-throughput single-cell TCR profiling is ideal because it provides a comprehensive view of T-cell heterogeneity and plasticity. However, since immune cell populations are complex and heterogeneous, it is also critical to dissect the cell types present in the profiled population to get a full understanding of the immune response. Towards this end, whole transcriptome analysis (WTA) can be used to aid in the identification of the different cell types—and even subtypes—present within a sample. We have developed a protocol (Figure 1) compatible with the ICELL8 Human TCR a/b Profiling and ICELL8 cx Human TCR a/b Profiling workflows that allows T-cell receptor (TCR) clonotype determination and 5'-end differential expression analysis from the same cell.

Results

Gene detection in different cell lines

For the experiments described below, 5'-DE libraries were generated for T cells and peripheral blood mononuclear cells (PBMCs) dispensed with the ICELL8 Single-Cell System using the 5'-DE protocol developed for the ICELL8 Human TCR a/b Profiling workflow (Figure 1). This method processes a portion of the barcoded full-length cDNA generated from oligo-dT priming during the on-chip RT-PCR to create libraries for 5' DE using Nextera® tagmentation and amplification of the 5'-end barcodes to create a whole transcriptome library. The on-chip cDNA amplification produces enough product to process another portion for clonotyping analysis in parallel. The same cDNA can also be processed using two rounds of gene-specific PCR to amplify cDNA sequences corresponding to the variable regions of *TCRa* and *TCRb* transcripts as described in our TCR profiling technical note.

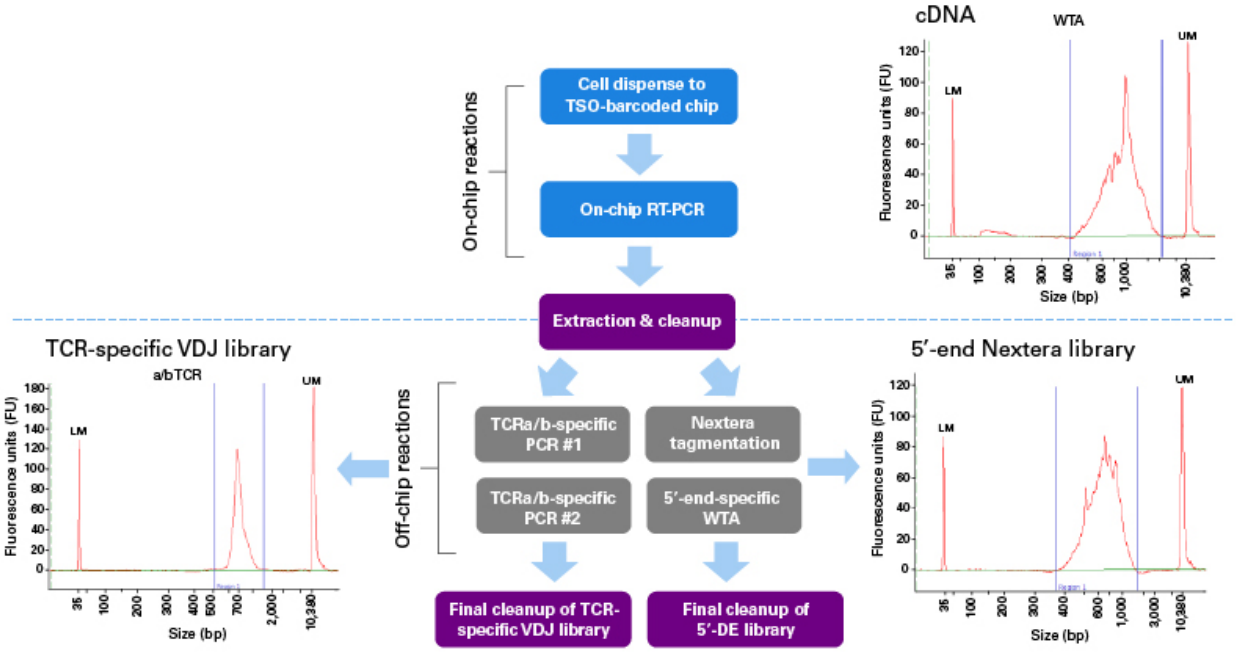

Figure 1. Generating 5'-DE libraries using the ICELL8 and ICELL8 cx Human TCR Profiling workflows. Top. After dispensing of samples (cells, positive control, and negative control) onto the ICELL8 TCR Chip, cDNAs were synthesized via oligo-dT priming on-chip. A Bioanalyzer trace from purified full-length cDNA shows an average cDNA amplicon length of 964 bp. **Bottom.** Following extraction of the cDNA from the chip, a portion of the cDNA was used for selective amplification of the *TCRa* and *TCRb* sequences of the TCR variable regions generating a library with broad peaks between ~650 to 1,150 bp and maximal peaks in the range of ~700 to 900 bp (see our TCR profiling technical note for full details). Another portion of the cDNA was processed using a Nextera XT DNA Library Preparation kit to enrich for cDNA derived from mRNA 5' ends. The 5'-end Nextera library shows an average size of ~700 bp. Peaks labeled "LM" and "UM" correspond to DNA reference markers.

Following sequencing of the pooled single-cell 5'-DE libraries, we assessed the number of reads obtained per barcode (Figure 2). Taking advantage of the ICELL8 system's ability to include controls, 15 negative controls containing all the reaction components except the sample were included as part of this experiment. This data was then used to set a confident read threshold to ensure high-quality data for the experimental samples. Pooled single-cell 5'-DE libraries after Nextera (Figure 1) were sequenced on a NextSeq® system to generate an average of 30K reads per cell. At an average sequencing depth of 30K reads per cell, we detected a median of ~1,150 genes in the PBMCs and T cells. Reads from the positive control RNA are well aligned with the reads for the T-cell samples and show good separation from the negative controls which are representative of background noise.

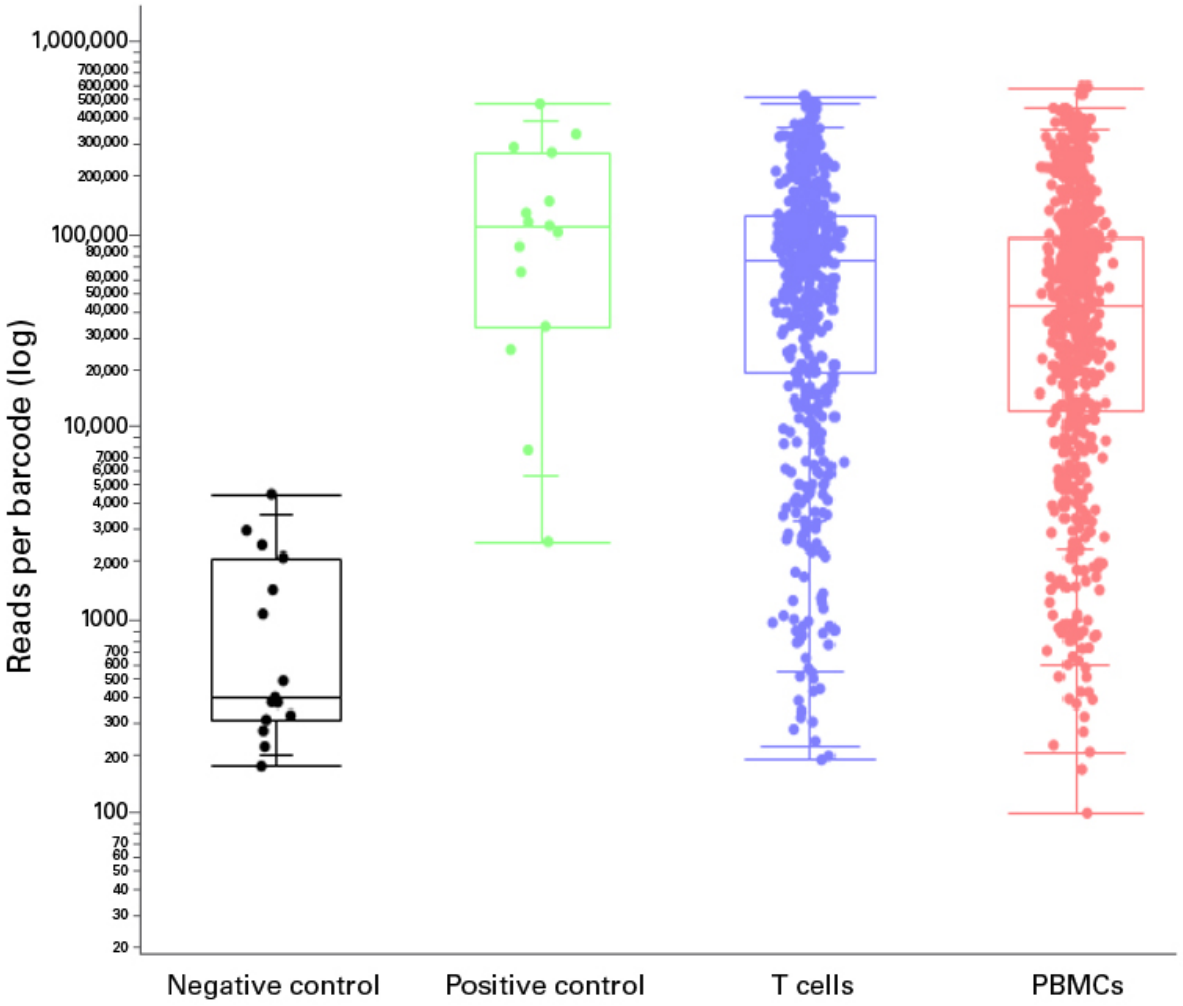

Figure 2. Single-cell RNA-seq profiling of T cells and PBMCs showing reads per barcode (cell). Each dot in the box plot represents the number reads for a given cell. For this data set, 15 negative control wells, 15 positive control wells (Jurkat total RNA), 530 T cells, and 590 PBMCs were processed and sequenced.

Assessment of cell types present within a PBMC sample

We next confirmed that we could distinguish various cell types present within a PBMC sample, using the sequencing data obtained from single-cell 5'-DE libraries. Principal component analyses were performed based on the top 500 most variable genes. Interestingly, the tSNE plot, based on the top 500 expressed genes (Figure 3), identified four main clusters. Notably, in the largest cluster, there is representation of both the PBMC and T-cell samples. This overlap is expected given that T cells can make up an estimated 70–80% of PBMC populations.

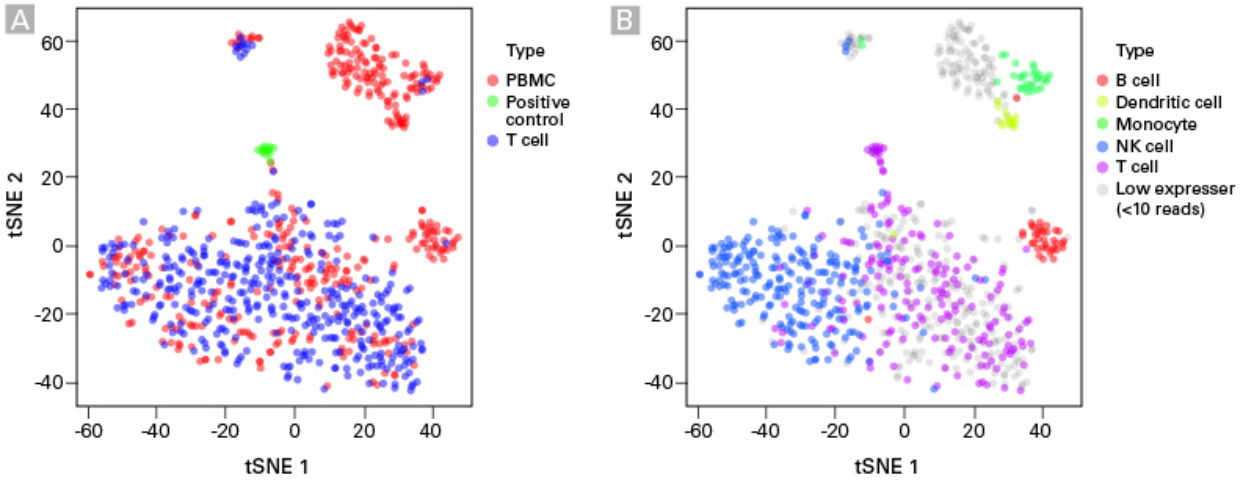

Figure 3. Clustering of T cells and PBMCs. Panel A. tSNE based on PBMC, T cell, and positive control samples with greater than 10K reads. Expression was normalized by the median coverage across cells and natural log transformed. Clustering was based on the top 500 expressed genes, resulting in four main clusters. **Panel B.** Individual cells were classified by calculating the maximum expression across a panel of expression markers for each of the cell types listed in the figure legend. Cells with less than 10 total reads aligned to any cell type within the panel were classified as low expressers. This classification scheme highlights the major cell types associated with each cluster in the tSNE plots.

In humans, the majority of PBMCs are lymphocytes (approximately 70–90% of total) with additional populations of monocytes and dendritic cells (~10–30% and 5–20% of all PBMCs, respectively). The lymphocyte population is further comprised of T cells (70–85%), B cells (5–20%), and NK cells (5–20%). We used different cell-specific markers based on published data (Palmer et al. 2006), to identify various populations of cells present within the PBMC sample (Figures 4). Consistent with reporting in the literature, T cells made up the largest percentage of the PBMCs, and the location of this cluster overlaps with the negatively selected T cells that were also profiled in these experiments. Furthermore, dendritic cells were the rarest cell type within the PBMC sample. We also identified B cell, monocyte, and NK cell populations using cell-specific markers expressed in these cell types.

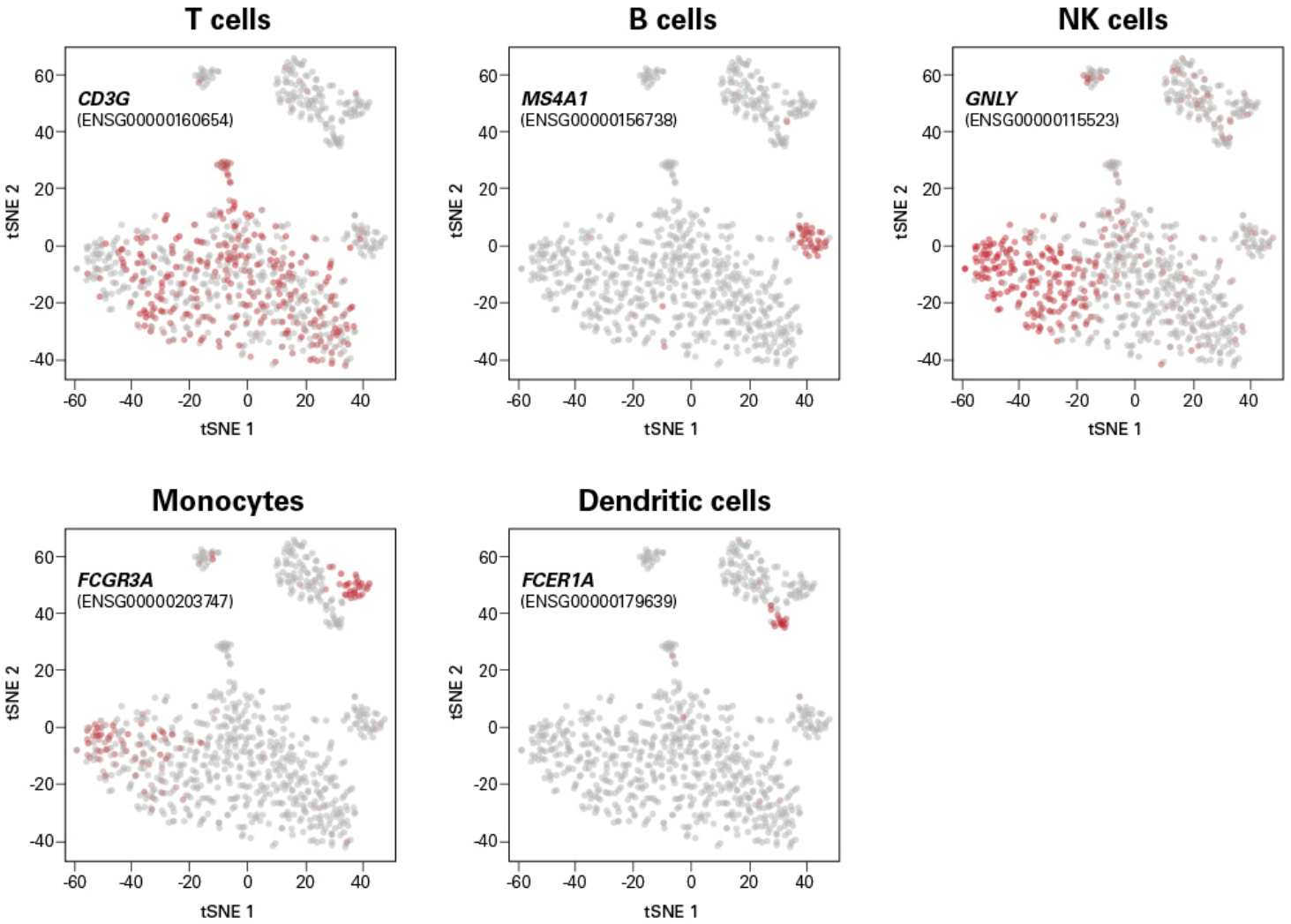

Figure 4. Identifying cell types in the PBMC population. Cell type clusters within the PBMC sample were identified based on known cell-type markers for T cells (*CD3G*), B cells (*MS4A1*), NK cells (*GNLY*), monocytes (*FCGR3A*), and dendritic cells (*FCER1A*).

Conclusions

The human TCR a/b profiling workflow with 5' differential expression for the ICELL8 systems can be used to generate Illumina sequencing libraries from thousands of single immune cells for the determination of TCR αß pairing as well as cell type information. This method is a highly sensitive and economical approach to identify clonotypes and cell types by using 5'-DE and TCR-specific priming on the same cDNA. The combination of this chemistry and the automated ICELL8 Single-Cell System or ICELL8 cx Single-Cell System enables profiling of >1,000 cells showcasing the general utility and scalability of this approach for studies investigating paired TCR clonotype diversity. Furthermore, the ability to set up positive and negative controls generates greater confidence in the data, especially when working with complex samples.

Methods

Nextera libraries for 5'-end differential expression analysis were generated using the 5'-DE workflow as described in the ICELL8 Human TCR a/b Profiling User Manual. For a positive control, Control Jurkat Total RNA (Takara Bio) was used. Isolated PBMCs (AllCells) were thawed in RPMI and washed once in media before staining the cells. T cells were isolated from whole blood using the EasySep Direct Human T Cell Isolation Kit (STEMCELL Technologies). Isolation was performed per manufacturer's recommendation. For sequencing, the final library was diluted to 1.8 pM, including a 20% PhiX Control v3 (Illumina) spike-in for sequencing. Sequencing was performed on an Illumina NextSeq sequencer using the 150-cycle NextSeq 550 System mid-output kit (Illumina) with paired-end, 2 x 75 base pair reads.

References

Palmer, C., Diehn, M., Alizadeh, A. A. & Brown, P. O. Cell-type specific gene expression profiles of leukocytes in human peripheral blood. *BMC Genomics* **7,** 115 (2006).

Related Products

Cat. #	Product	Size	License	Quantity	Details
640048	ICELL8® Collection Kit	1 Pkg		*	
The ICELL8 Collection Kit consists of a fixture, tube, and film that allow users to spin down the contents of an ICELL8 chip to collect the contents of processed chips (e.g., extracted cDNA) into a tube. Documents | Components | Image Data					
640197	ICELL8® cx Loading Kit	Each		*	
640200	ICELL8® cx TCR Chip	1 Chip		*	
640179	ICELL8® Human TCR a/b Profiling - Indexing Primer Set	1 Chip		*	
640180	ICELL8® Human TCR a/b Profiling - Indexing Primer Set	5 Chips		*	
640181	ICELL8® Human TCR a/b Profiling - Indexing Primer Set	10 Chip		*	
640182	ICELL8® Human TCR a/b Profiling Reagent Kit	Each		*	
640109	ICELL8® Loading Kit	1 Pkg		*	
640178	ICELL8® TCR Chip	1 Chip		*	

Add to Cart

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999