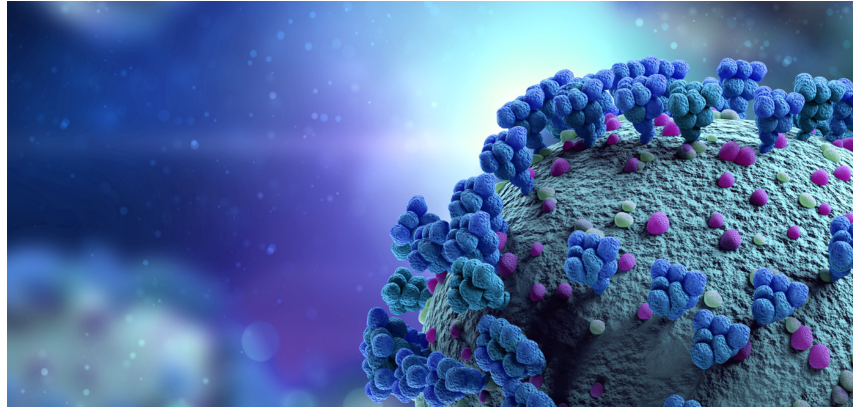
Single-cell T cell receptor repertoire analysis of sorted γδ T cells using the BD Rhapsody™ TCR/BCR Multiomic Assay

Features

- Characterization of γδ T cell clonal enrichment and full-length T cell receptor sequences using the BD Rhapsody™ TCR/BCR Multiomic Assay
- Co-staining cells with both fluorescent antibodies and BD® AbSeq Antibody-Oligo Conjugates enables cell sorting followed by cell surface protein profiling of single cells with BD® AbSeq Reagents
- Cell sorting in combination with the BD Rhapsody™ Single-Cell Analysis System facilitates deep characterization of rare immune cell populations using a multiomic approach that combines simultaneous profiling of single-cell mRNA, cell surface proteins using AbSeq and T cell receptors



Identification of paired, full-length γδ T cell receptor sequences and in-depth analysis of γδ T cell receptor clonal expansion in a latent tuberculosis (TB) donor

T cells are a distinct immune cell type that possess the ability to specifically recognize antigens and protect against cancer and invading pathogens. This functional flexibility is driven by the specific T cell receptor (TCR), a transmembrane heterodimer consisting of either αβ or γδ chains. In humans, γδ T cells are of low abundance in the body and constitute a small subset of T cells in blood. Despite their rarity, they play a critical role in immune surveillance against tumors and immune responses to intracellular pathogens such as *Mycobacterium tuberculosis* M. tb. Selective γδ T cell populations may expand in the peripheral blood of TB-infected donors, playing a crucial role in anti-mycobacterial immunity. Therefore, it is important to characterize the clonality and diversity of the γδ T cell repertoire and identify the sequence of the paired γδ TCR chains to elucidate their function in the immune response to M. tb infection.

In this study, a comprehensive workflow was utilized in which rare γδ T cells were enriched from a latent TB donor using the BD FACSAria™ Fusion Cell Sorter followed by a single-cell multiomic assay using the BD Rhapsody™ Single-Cell Analysis System. Using the BD Rhapsody™ TCR/BCR Multiomic Assay in conjunction with single-cell gene and AbSeq marker expression, γδ T cells were identified and their clonotype expansion was examined in the latent TB donor, compared to a healthy donor. Additionally, the paired full-length sequence of the top enriched γδ T cell clonotypes was identified.

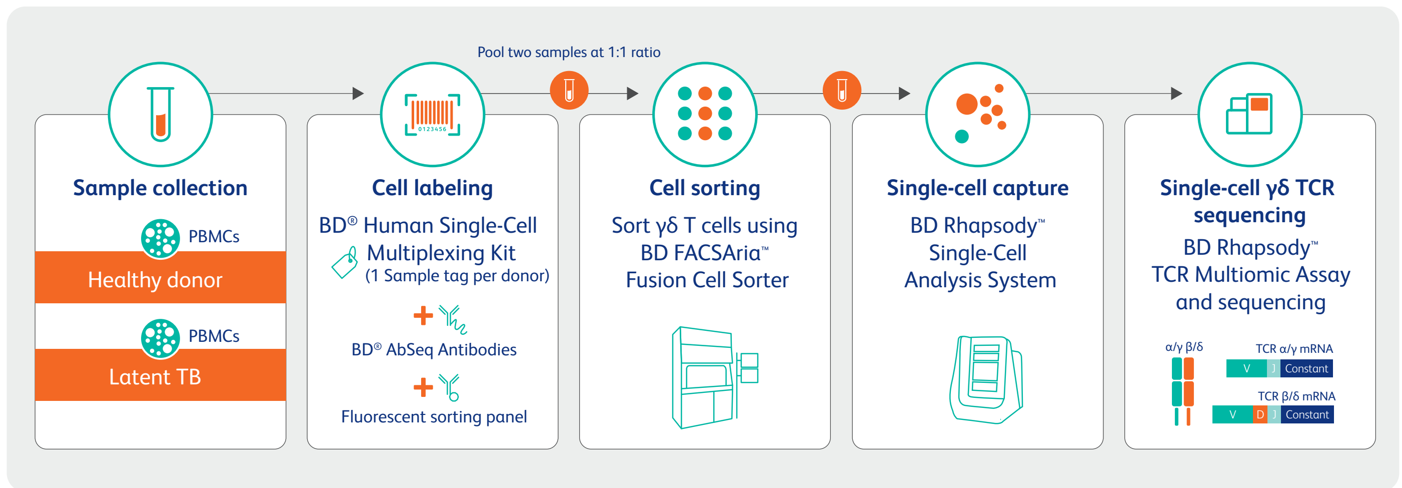


Figure 1. Experimental overview of cell staining and sorting followed by single-cell analysis using the BD Rhapsody™ Single-Cell Analysis System.

Peripheral blood mononuclear cells were isolated from either a healthy or latent TB donor. Cells from each sample were co-stained with a unique sample multiplexing antibody, five fluorescent antibodies (Table 1, left), and an 18-plex BD® AbSeq Antibody-Oligo Panel (Table 1, right) prior to cell sorting. Different sample multiplexing antibodies allowed the two samples to be distinguished in downstream analysis. After staining, the two samples were combined at a 1:1 ratio for sorting $CD3^+CD4^-CD8^-$ γδ T cells using the BD FACSAria™ Fusion Cell Sorter. Approximately 20,000 sorted cells were loaded onto the BD Rhapsody™ Single-Cell Analysis System for single-cell capture. TCR, BD® AbSeq Antibody-Oligo Panel, targeted mRNA (BD Rhapsody™ Human Immune Response Panel) and sample tag libraries were prepared for sequencing.

Table 1. List of fluorescent antibodies and BD® AbSeq Antibody-Oligos used for the study

Five-color cell sorting panel

Marker	Fluorochrome	Clone
Live/dead	V500 (dump gate)	
CD19		
CD14		
CD8*	BV650	RPA-T8
CD3*	Alexa Fluor™ 700	RPA-T4
CD4*	APC-eFluor 780	UCHT1

BV = BD Horizon Brilliant™ Violet
V = BD Horizon™ Violet

18-plex BD® AbSeq Panel

Marker	Clone
Vδ2 TCR	B6
γδ TCR	11F2
αβ TCR	IP26
HLA-DR	G46-6
CD314 (NKG2D)	1D11
CD69	FN50
CD62L	SK11
CD56	NCAM16.2
CD45RO	UCHL1
CD45RA	5H9
CD272 (BTLA)	J168-540
CD27	M-T271
CD152 (CTLA4)	BNI3
CD137	4B4-1
CCR7	2-L1-A
CD8*	SK1
CD4*	RPA-T4
CD3*	UCHT1

*The same marker of AbSeq antibodies and fluorescent antibodies were used. This strategy did not compromise signal integrity as determined by a flow proxy assay (see https://star-protocols.cell.com/protocols/1105 for a detailed protocol).

UMAP analysis was performed using combined mRNA and AbSeq protein expression on sorted γδ T cells. UMAP dimensional reduction revealed cells from the healthy donor were distributed into three clusters while cells from the latent TB donor were located mainly in one larger cluster (Figure 2). Analysis of γδ TCR, CD4 and CD8 protein expression confirmed the successful sorting of $CD3^{+}CD4^{-}CD8^{-}$ γδ T cells (Figure 2). Additionally, concordant expression of CCR7 and CD62L protein markers and reverse expression between CD45RA protein and CD45RO protein were observed, which is consistent with T cell functional status (Figure 2).

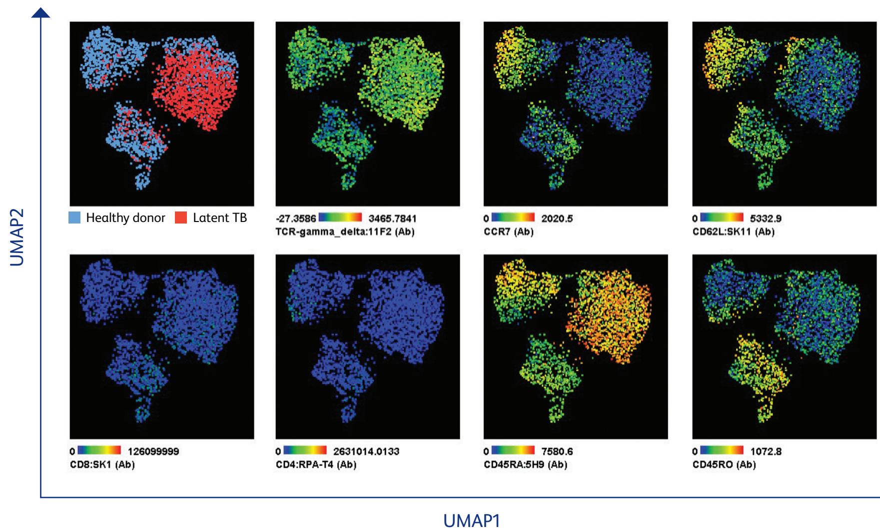


Figure 2. Single-cell analysis on sorted γδ T cells.

UMAP analysis on sorted γδ T cells shows the distribution of the healthy and latent TB donors. Examination of TCR γδ, CD4 and CD8 protein expression confirms the identity of the sorted cells. CCR7 and CD62L showed a similar protein expression pattern and reverse expression between CD45RO and CD45RA was observed. A comparable number of cells were recovered from both donors, confirming quality of sample preparation.

Additional analysis of γδ TCR V and J gene usage showed differences in their ratios between the two donors (Figure 3A). Chord diagrams illustrated the pairing of V and J genes of γδ TCR chains in the two samples with notable differences between the two samples (Figure 3B). Lastly, we examined the top enriched clonotypes in the latent TB donor, all of which were absent in the healthy donor, suggesting those clones may respond to the TB antigen. With the BD Rhapsody™ TCR/BCR Multiomic Assay, we could identify the paired full-length nucleotide and amino acid (not shown) sequences of the topmost enriched clonotypes (Figure 3C).

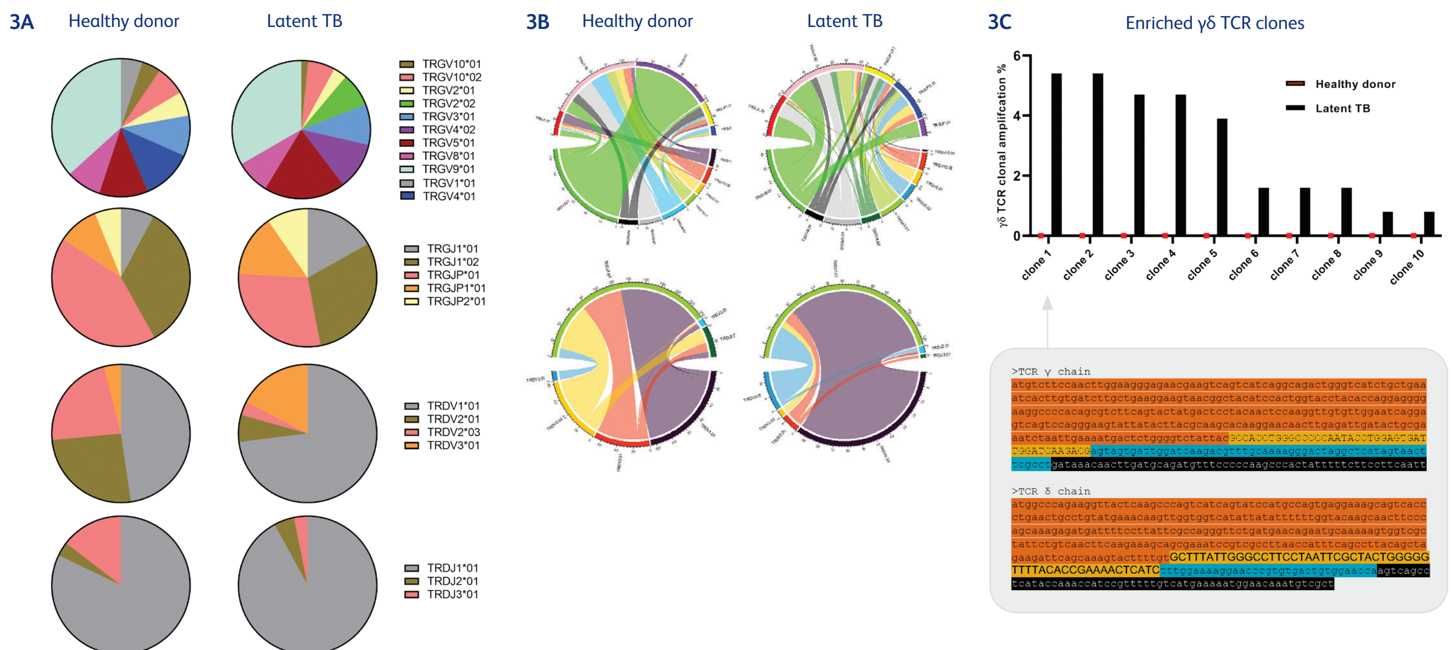


Figure 3. Single-cell TCR analysis on sorted γδ T cells.

A. Distribution of V and J gene usage of γδ T cell receptors in healthy and latent TB donors. **B.** The distribution of TCR γδ clonotypes depicted by chord diagrams. Each arc represents a V or J gene segment and is scaled lengthwise based on the relative proportion. Each chord represents a unique V-J combination and is weighted according to the relative abundance of that combination in the dataset. **C.** The frequency of the top 10 γδ TCR pairs in the latent TB donor in comparison with the healthy donor. The sequence of the top expanded clone is shown color-coded as follows: V (red highlight), CDR3 (green highlight) J (blue highlight) and part of the constant region (black highlight).

In summary, the full-length paired γδ TCR sequence and clonal enrichment were successfully detected in a biological sample using the BD Rhapsody™ TCR/BCR Multiomic Assay. This enrichment can be easily missed using other single-cell immune profiling kits due to the absence of γδ TCR primers. An integrative analysis of mRNA, AbSeq and TCR allows users to interrogate the transcriptome, surface protein markers and TCR repertoire at the single-cell level.

Products utilized in this study	
Instruments	**Cat. No.**
BD Rhapsody™ Single-Cell Analysis System	633701
BD Rhapsody™ Express Single-Cell Analysis System	633702
Reagents	**Cat. No.**
BD® Hu Single Cell Sample Multiplexing Kit	633781
BD Rhapsody™ Cartridge Kit	633733
BD Rhapsody™ Enhanced Cartridge Reagent Kit	664887
BD Rhapsody™ cDNA Kit	633773
BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit – 4 pack	665913
BD Rhapsody™ Immune Response Panel HS	633750
BD Rhapsody™ TCR/BCR Amplification Kit	665345
BD® AbSeq Antibody Panel	**Cat. No.**
BD® AbSeq Oligo Mouse Anti-Human Vδ2 TCR	940297
BD® AbSeq Oligo Mouse Anti-Human γδ TCR	940365
BD® AbSeq Oligo Mouse Anti-Human TCRαβ	940074
BD® AbSeq Oligo Mouse Anti-Human HLA-DR	940010
BD® AbSeq Oligo Mouse Anti-Human CD314 (NKG2D)	940061
BD® AbSeq Oligo Mouse Anti-Human CD69	940019
BD® AbSeq Oligo Mouse Anti-Human CD62L	940387
BD® AbSeq Oligo Mouse Anti-Human CD56	940007
BD® AbSeq Oligo Mouse Anti-Human CD45RO	940022
BD® AbSeq Oligo Mouse Anti-Human CD45RA	940263
BD® AbSeq Oligo Mouse Anti-Human CD272 (BTLA)	940105
BD® AbSeq Oligo Mouse Anti-Human CD27	940018
BD® AbSeq Oligo Mouse Anti-Human CD152	940034
BD® AbSeq Oligo Mouse Anti-Human CD137	940055
BD® AbSeq Oligo Mouse Anti-Human CCR7 (CD197)	940394
BD® AbSeq Oligo Mouse Anti-Human CD8	940305
BD® AbSeq Oligo Mouse Anti-Human CD4	940304
BD® AbSeq Oligo Mouse Anti-Human CD3	940307

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