



Single-cell immune profiling via hybridization-capture enrichment and Oxford Nanopore sequencing



Scott Hickey¹, Xiaoguang (David) Dai¹, Sissel Juul¹

¹Oxford Nanopore Technologies Inc., New York, NY

Abstract

Full-length sequencing of T-cell receptor (TCR) transcripts at single-cell resolution provides comprehensive clonotype analysis for adaptive immune repertoire profiling. Fragmentation of transcripts in short-read sequencing workflows limits TCR annotation and clonotype recovery. Current TCR enrichment methods rely on nested PCR to recover cell barcodes, UMIs and V(D)J regions located near the 5' ends of the TCR transcripts^{1,2}. Here, using human PBMCs, we demonstrate a hybridization-capture workflow that enables the enrichment and sequencing of full-length TCR transcripts from a 10x Genomics 3' single-cell cDNA library. Using a compact biotinylated probe panel, TCR reads are enriched >1,000-fold relative to a matched unenriched dataset. The enriched dataset recovers 4,694 unique functional TCR clonotypes, spanning TRA, TRB, TRD and TRG chains across 2,432 cell barcodes. Subsampling shows that clonotype recovery approaches saturation using only a fraction of the output from a PromethION™ flow cell, justifying sample multiplexing without substantial loss of repertoire complexity. Enriched single-cell TCR reads support paired-chain reconstruction and clonotype-network analysis. We map the expanded T-cell clones onto the transcriptomic landscape, where gene expression analysis identifies a cytotoxic CD8+/TEMRA (terminally differentiated effector memory cells re-expressing CD45RA) program. Full-length Oxford Nanopore reads also reveal differential CD45 (*PTPRC*) isoform usage between expanded T-cell clones and singletons.

Methods

Oxford Nanopore single-cell immune profiling via hybridization-capture enrichment

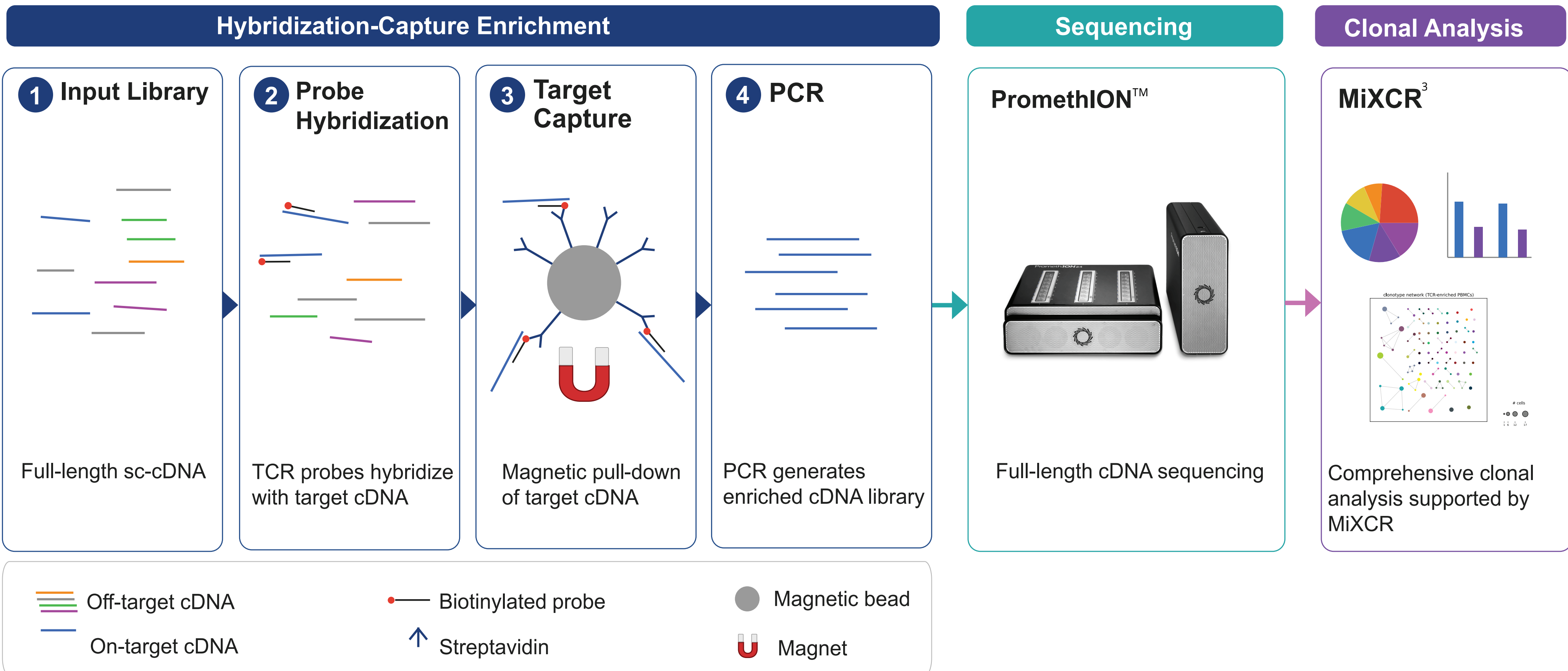


Figure 1. Hybridization-capture-based TCR enrichment and sequencing workflow.

Full-length TCR cDNA transcripts are captured from a 10x Genomics 3' GEM-X V4 human PBMC library using a compact biotinylated TCR probe panel⁴, followed by streptavidin magnetic bead pull-down and PCR amplification. Both the unenriched control library and TCR-enriched library undergo Oxford Nanopore library preparation and are sequenced on separate PromethION flow cells. Clonotype reconstruction is performed using MiXCR with Oxford Nanopore presets.

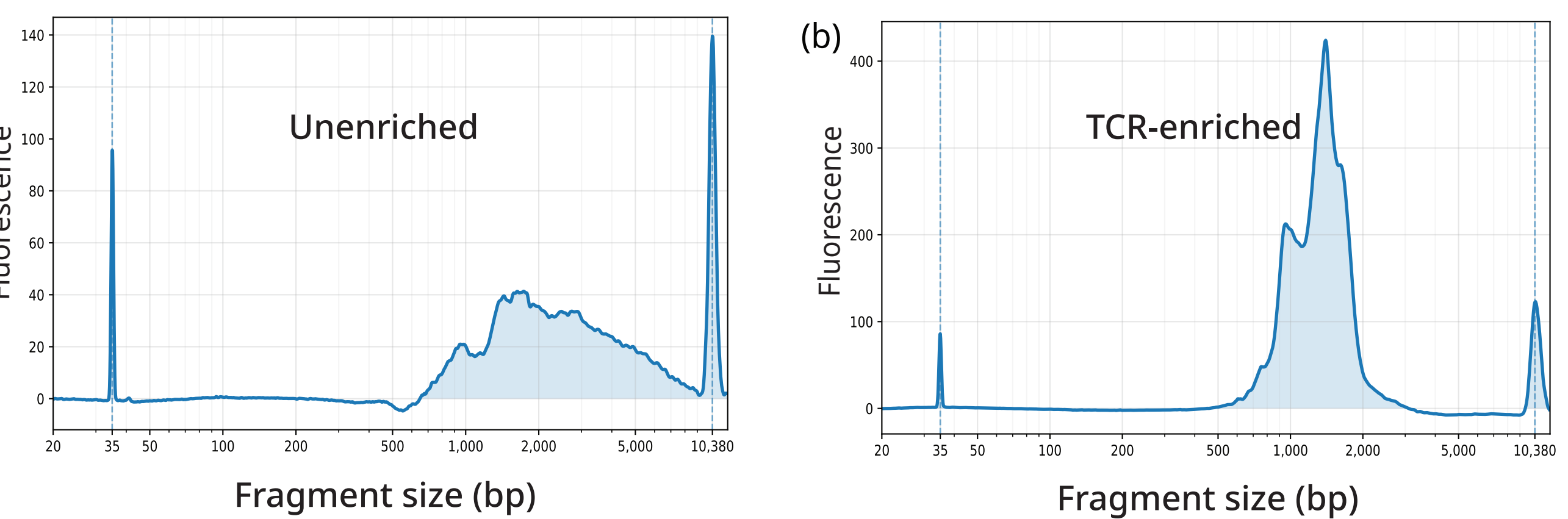


Figure 2. Bioanalyzer traces of the unenriched (a) and TCR-enriched (b) scRNA-seq libraries.

Following enrichment, the cDNA profile shifts to a sharper distribution, reflecting an increased abundance of full-length TCR transcripts with an expected range around 1.0 to 1.5 kb. This shift may vary across input single-cell libraries depending on the relative abundance of TCR transcripts. Sequencing performance is maintained following hybridization capture (Table 1).

Table 1	Throughput (Gb)	Read Counts (M)	Read N50 (Kb)
Unenriched	112	99	1.24
TCR-enriched	126	112	1.25

Enrichment improves TCR read counts, chain pairing & clonotype recovery

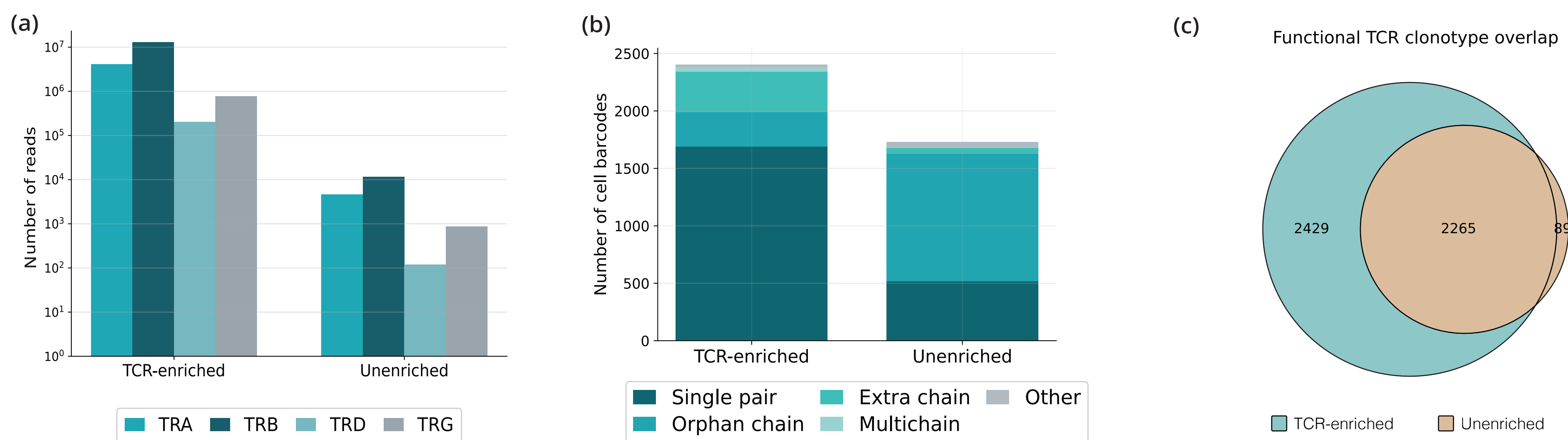


Figure 3. Hybridization-capture enrichment increases TCR read counts, improving chain pairing and clonotype detection.

(a) Bars show raw read counts assigned to each TCR chain in TCR-enriched and unenriched libraries; enrichment results in >1,000-fold more on-target reads per PromethION flow cell. (b) Stacked bars show the number of cell barcodes in each pairing class for TRA and TRB chains. Over 70% of cell barcodes contain one pair of TRA+TRB. In contrast, the unenriched dataset shows 64% of cells with only a single detected TRA or TRB chain, reflecting insufficient sequencing depth. (c) Clonotype comparison shows a large overlap between unenriched and enriched datasets. The enriched library recovers 4,694 unique functional clonotypes compared with 2,354 in the unenriched library, representing a 2x increase.

Subsampling analysis supports sample multiplexing

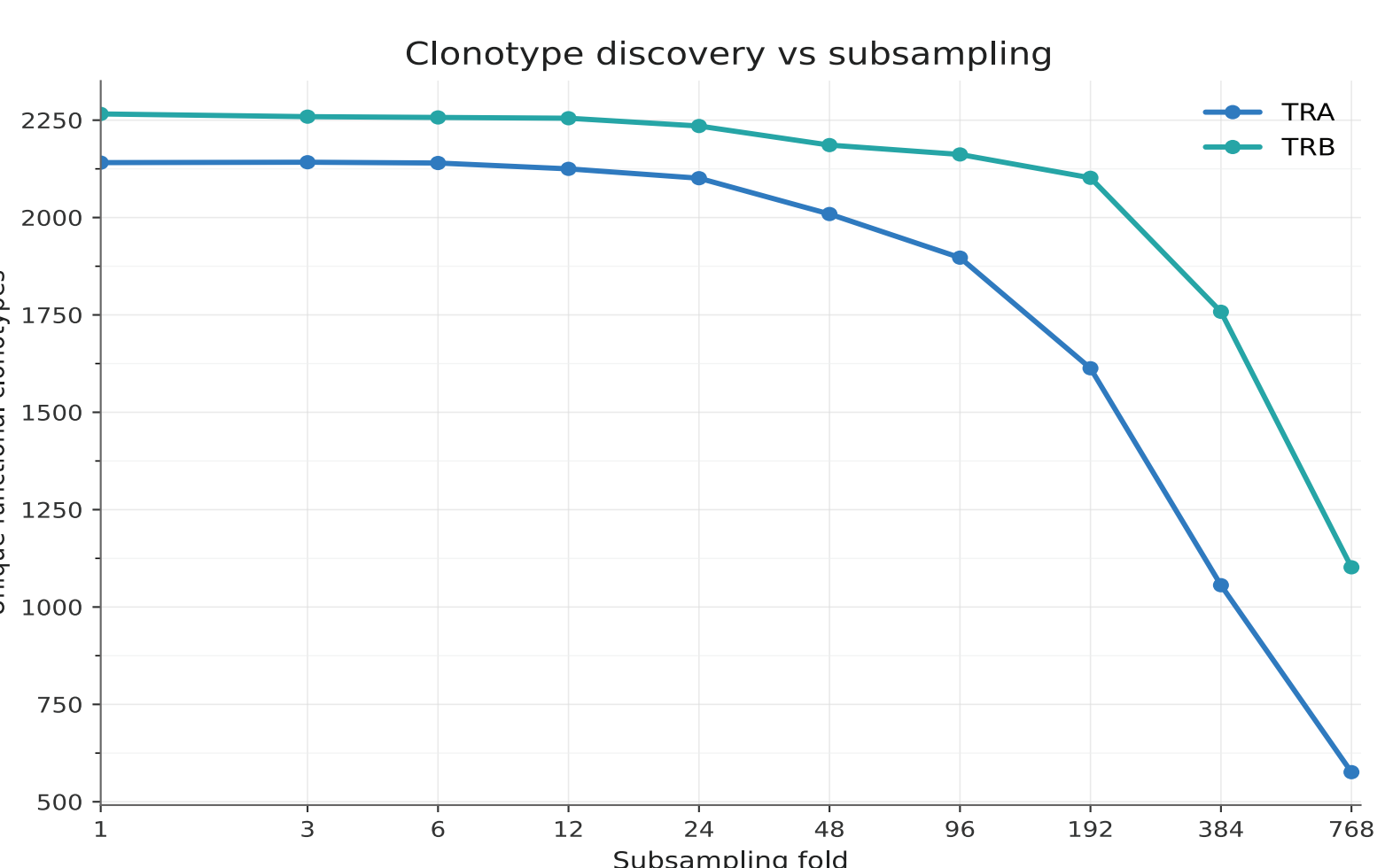


Figure 4. Subsampling analysis supports sample multiplexing on a PromethION flow cell.

To assess the sequencing depth needed for clonotype recovery, the enriched dataset is subsampled and unique functional TRA and TRB clonotypes are quantified at each depth. Clonotype discovery remains essentially stable through 12-fold subsampling, with 2,125 of 2,141 TRA clonotypes (99.3%) and 2,255 of 2,266 TRB clonotypes (99.5%) still recovered at one-twelfth of the original depth. Even at 24-fold subsampling, more than 98% of clonotypes are retained for both chains. These results indicate that enriched libraries approach clonotype saturation using only a small fraction of the output from a PromethION flow cell, supporting multiplexing samples per run while preserving robust repertoire profiling.

Full-length TCR sequences resolve clonotype networks

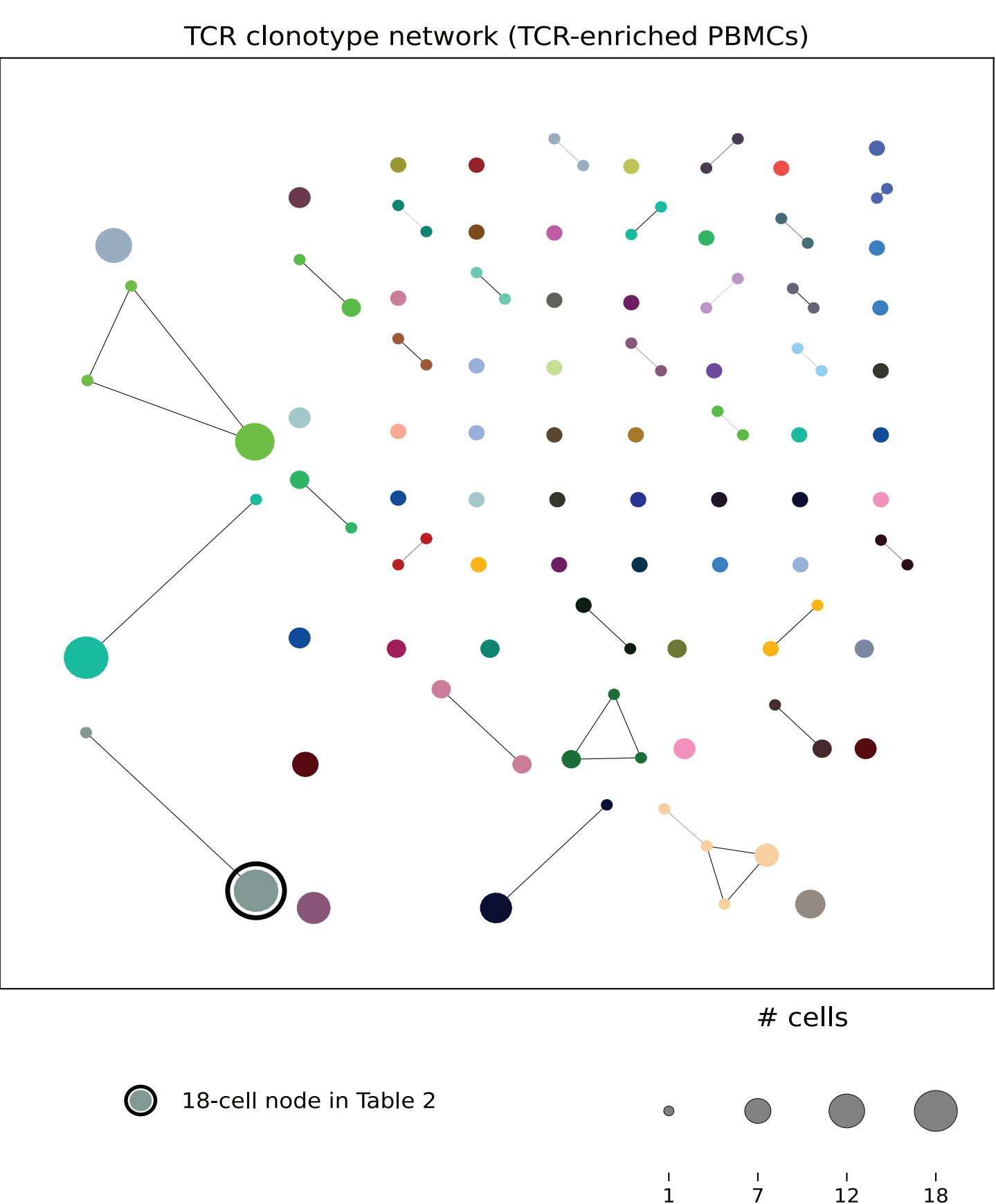


Figure 5. Increased recovery of full-length TCR sequences through enrichment enables downstream analysis of clonotype relationships.

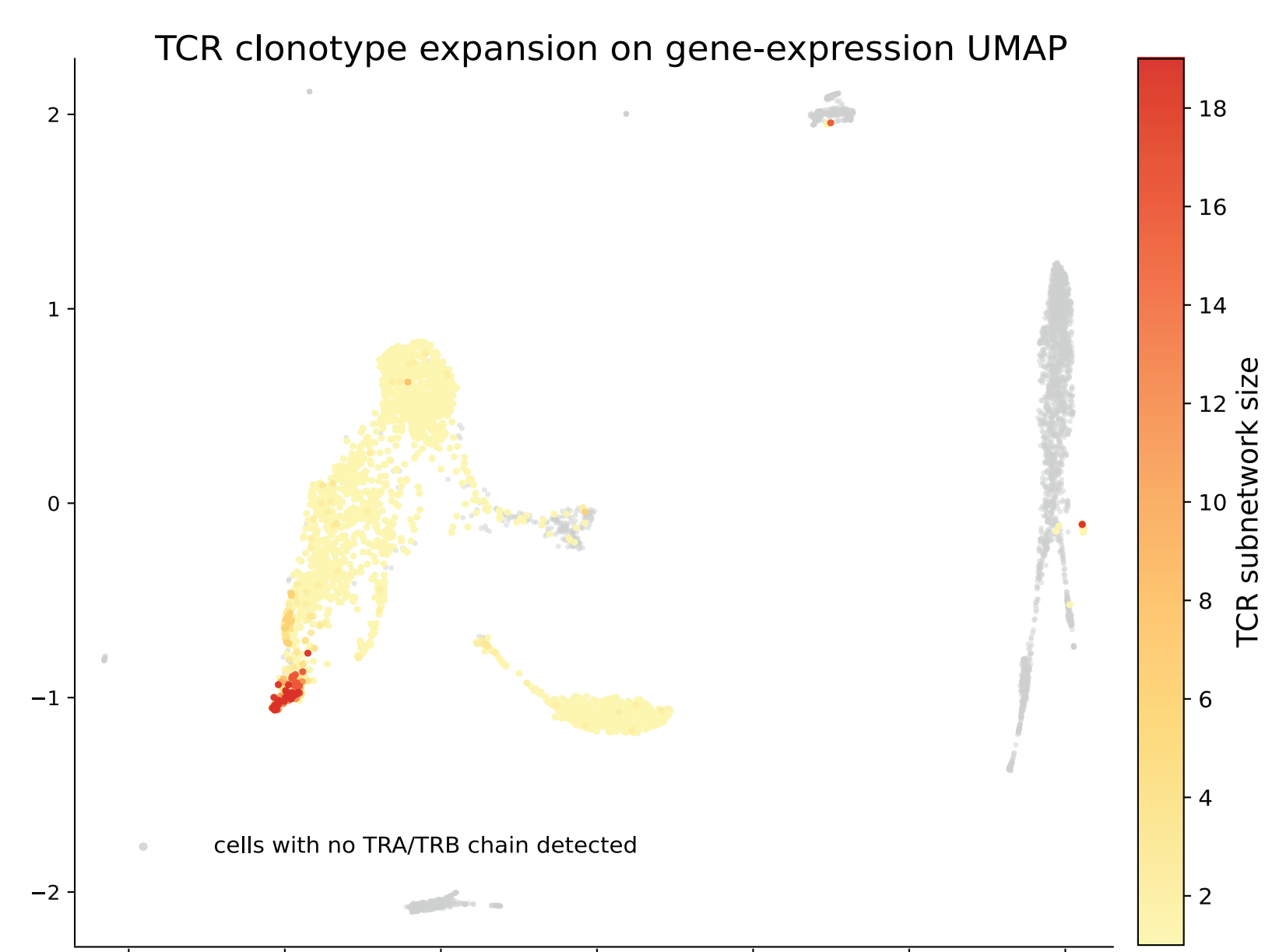
In this ~5,000 cells human PBMC library, we detect 2,402 T-cells with at least one TRA or TRB chain identified. CDR3 amino acid distances across TRA and TRB chains are computed in Scirpy using the TCRdist metric. A clonotype network is constructed from clonotypes represented by at least two cells. In Fig. 5, each node represents a group of cells sharing the same receptor-chain configuration and identical CDR3 amino acid sequences for each chain. Edges connect nodes with related receptor configurations based on CDR3 amino acid similarity. Full-length cDNA reads enable recovery of V(D)J and C (constant) gene assignments as well as CDR3 amino acid sequences, which is critical for accurate clonotype reconstruction and the study of T-cell functional diversity⁵. In Table 2, TRA and TRB chains are fully resolved for the largest node (18 T-cells) in the clonotype network.

Table 2	V call	D call	J call	C call	CDR3 amino acid
TRA	TRAV30	NA	TRAJ20	TRAC	CGTENDYKLSF
TRB	TRBV27	TRBD2	TRBJ2-3	TRBC2	CASSFNRLRATADTQYF

Expanded TCR clonotypes cluster within the transcriptomic landscape

Figure 6. Barcode-matched projection of the TCR clones onto the PBMC gene-expression UMAP shows discrete regions of clonal expansion.

All cell barcodes with a functional TRA and/or TRB chain detected in the enriched run are projected onto the unenriched gene expression map, constructed by wf-single-cell. Cells are colored by the size of the TCR node to which they belong, with warmer colors indicating larger clonal expansions; cells without a detected functional TRA/TRB chain are shown in grey. This view places receptor-defined clonal expansion within the broader transcriptomic landscape. It shows that expanded clonotypes are located in specific regions of the gene-expression map. In this PBMC sample, most TRA/TRB-positive cells are singletons, consistent with a diverse circulating T-cell repertoire. The largest expanded subnetworks form compact neighborhoods on the UMAP. This pattern suggests antigen-experienced or recently expanded T-cell populations.



Expanded TCR clonotypes are enriched for cytotoxic CD8+ & TEMRA programs

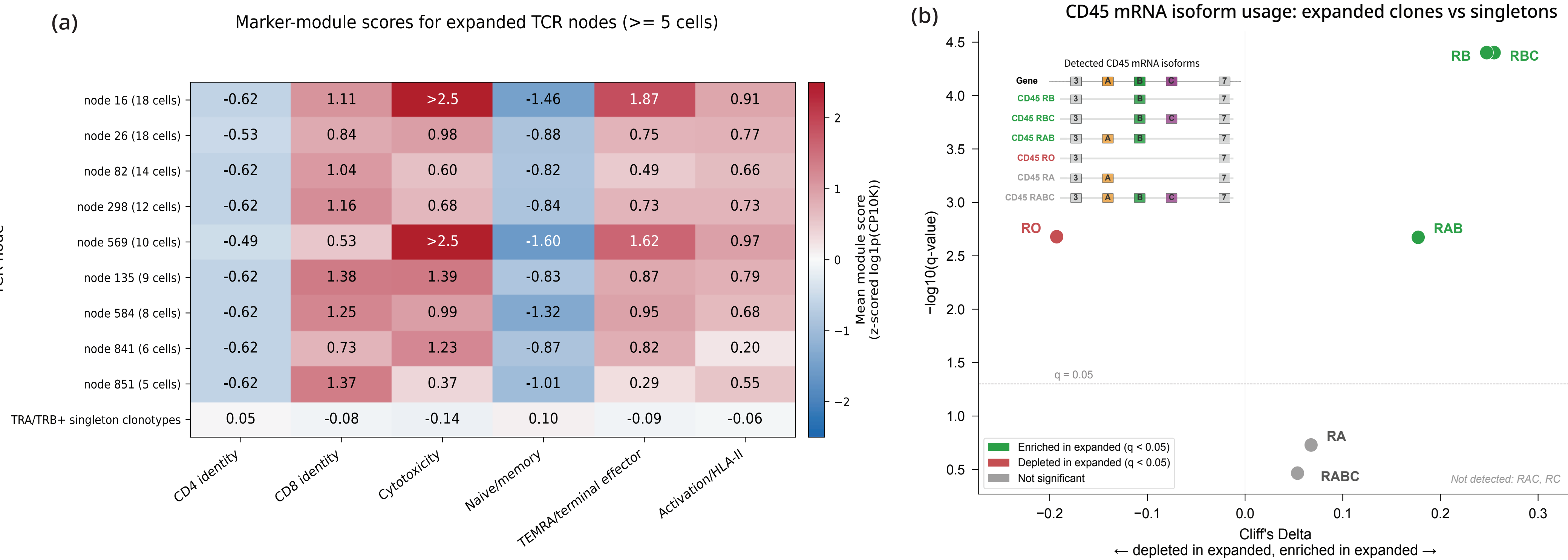


Figure 7. Expanded TCR clonotypes are enriched for cytotoxic CD8+ and TEMRA programs.

(a) Mean marker-module scores for expanded TCR nodes containing at least five cell barcodes are compared with singleton clonotypes. Expanded nodes show higher cytotoxicity, CD8 identity, TEMRA/TEM, and activation/HLA-II module scores but lower naive/memory and CD4 identity signals, supporting a cytotoxic CD8+/TEMRA interpretation of the expanded receptor-defined clones^{6,7}. (b) CD45 (*PTPRC*) isoform usage in expanded versus singleton clonotypes. Mean per-cell isoform fractions are compared across six isoforms detected by bambu⁸. Expanded clonotypes are depleted of isoform CD45 RO and enriched for isoforms CD45 RB, RBC, RAB, consistent with a CD8+/TEMRA phenotype⁹.

Conclusions

We present a workflow for full-length single-cell TCR profiling that combines hybridization-capture enrichment with Oxford Nanopore sequencing. Using a compact hybridization probe panel applied directly to 10x Genomics 3' single-cell cDNA libraries, this approach substantially increases recovery of TCR reads. The resulting gain in on-target depth improves paired-chain recovery and full-length TCR reconstruction and clonotype discovery. Gene expression and isoform analysis further link the TCR clonotypes to their cell states, all enabled by sequencing the same single-cell cDNA libraries (enriched and unenriched) on Oxford Nanopore PromethION flow cells.

References

- Luo et al. (2025) Clin Chem 71:274–285.
- Tu et al. (2019) Nat Immunol 20:1692–1699.
- <https://mixcr.com/>
- Beaulaurier et al. (2025) Genome Res 35:929–941.
- Singh et al. (2019) Nat Commun 10:3120.
- Lau et al. (2022) Nat Commun 13:4053.
- Yost et al. (2019) Nat Med 25:1251–1259.
- Chen et al. (2023) Nat Methods 20:1187–1195.
- Turk et al. (2024) Front Immunol 15:1285798.