Tumor reactivity assessment using clonal expression (TRACE) reveals heterogeneity of tumor reactive T cell populations across solid tumors

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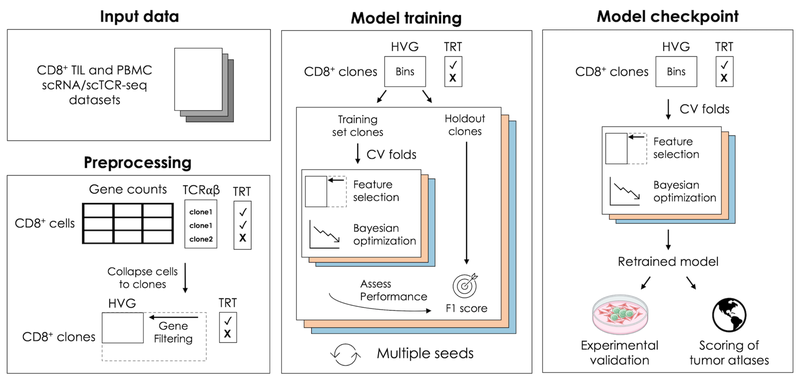
KSQ THERAPEUTICS

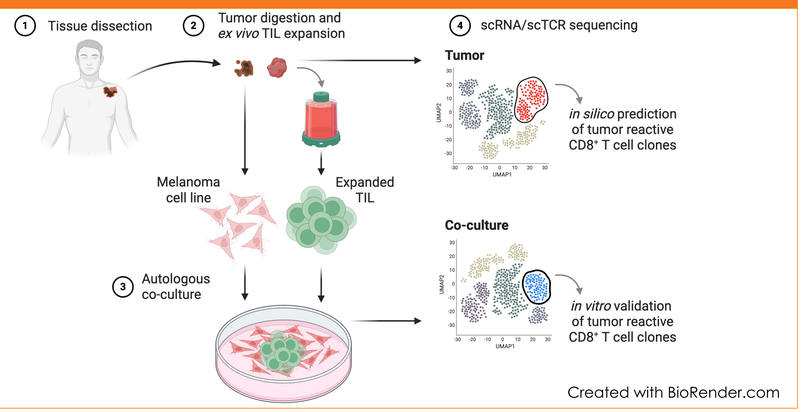
Background

- Tumor infiltrating lymphocytes (TIL) drive the anti-tumor activity of a broad class of immunotherapies, including CAR-T and *ex vivo* expanded TIL therapies
- The T cell receptor (TCR) repertoires present within TIL drug products are unique to each patient and include both T cells that recognize tumor antigens (Tumor Reactive T cells, or TRTs) and bystander T cells with specificity for other antigens
- TRT clonotypes are associated with an exhausted transcriptional state, enabling single-cell RNA sequencing (scRNA-seq)-based predictive models for TRTs using experimentally validated clone labels
- In this study, a clonotype-level CD8⁺ TRT classifier (TRACE) was built using an aggregated dataset of validated tumor reactive clonotypes and associated scRNA-seq data from multiple publications[1-9] that overcomes the limitations of training on a single dataset, donor, or indication
- TRACE does not require data preprocessing for training or prediction, enabling it to be easily re-trained or applied to new datasets to identify potential tumor-reactive clones

TRACE: clone-level TRT classifier built on scRNA/scTCR-seq datasets



TRACE validation using *ex vivo* expanded TIL/autologous tumor co-cultures



References

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2. Hanada *et al. Cancer Cell* (2022)
3. Lowery *et al. Science* (2022)
4. Meng *et al. Sci Transl Med* (2023)
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10. Zeng *et al. Nat Commun* (2025)
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12. Salcher *et al. Cancer Cell* (2022)
13. Chu *et al. Nat Cancer* (2024)

Figure 1: Clones predicted to be TRT by TRACE exhibit functional reactivity against an autologous melanoma cell line

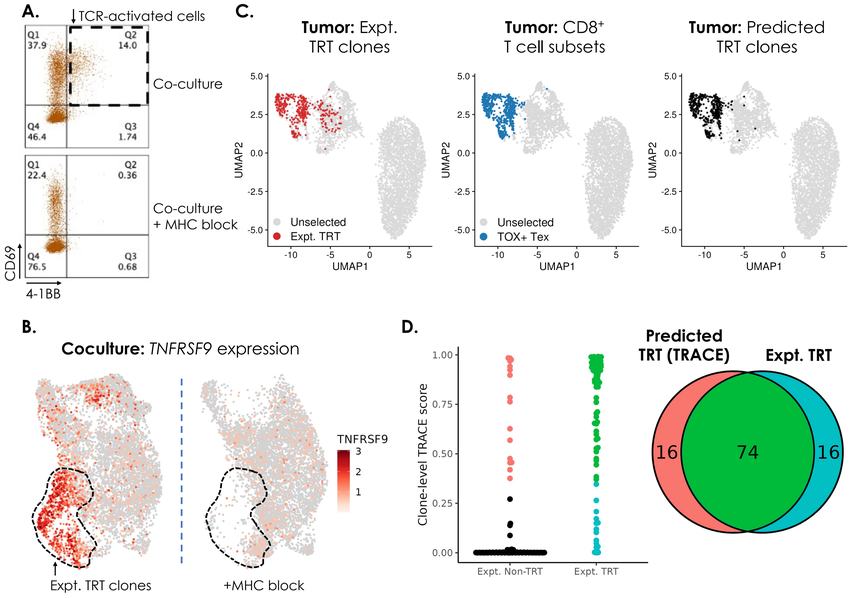
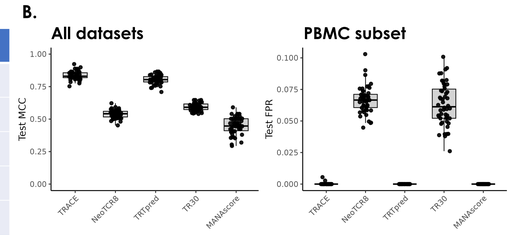


Figure 1. (A, B) *ex vivo* expanded TIL co-cultured with an autologous tumor cell-line exhibit MHC-mediated functional activation measured by cell-surface 4-1BB expression (A) and upregulation of *TNFRSF9* (encoding 4-1BB, shown on UMAP) (B) after 20 h co-culture. Expt. TRT, experimentally-verified TRT. **(C)** UMAPs showing location of Expt. TRT clones tracked to tumor starting material alongside location of *TOX*-expressing exhausted CD8⁺ T cells and putative TRT clones predicted by TRACE. **(D)** Summary of overlap between Expt. TRT clones and clones predicted to be TRT by TRACE.

Figure 2: TRACE exhibits comparable or superior performance to other tumor reactivity prediction methods

A.

TRT prediction method	Genes (Selection method)	Scoring / predictions
TRACE	50 genes (model tuning)	XGBoost
NeoTCR8 [3]	243 genes (Seurat FindMarkers)	scGSEA
TRTpred [6]	180 genes (edgeR + QLF)	Singscore*
TR30 [4]	30 genes (Seurat FindMarkers)	UCell
MANAscore [10]	3 genes (Authors' selection)	Custom code



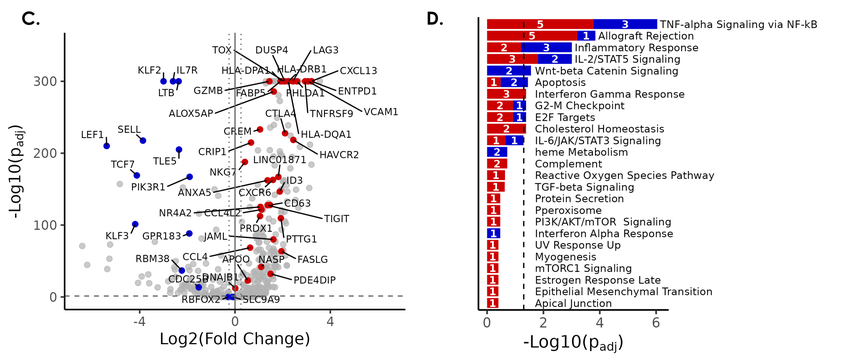


Figure 2. (A) Table with details of other TRT prediction methods implemented and benchmarked against TRACE. *For TRTpred, the final scoring method was not identified, so Singscore was used. **(B)** Model performance: test set Matthews Correlation Coefficient (MCC) of TRACE and other TRT methods across 50 seeds on test sets containing clones from all available datasets and False Positive Rate (FPR) on test sets subset for clones from PBMC datasets. **(C)** Volcano plot highlighting the 50 genes used by TRACE. Grey genes are other highly variable genes used to establish the bins but not used in the final model. **(D)** Bar plot with Hallmark pathways containing TRACE genes. On volcano plot, dashed horizontal line represents FDR = 0.05 and dashed vertical lines represent |log2(FC)| = 0.25. On bar plot, dashed vertical line represents FDR = 0.05.

Figure 3: TRACE precisely identifies antigen-experienced, exhausted CD8⁺ T cells across solid tumors

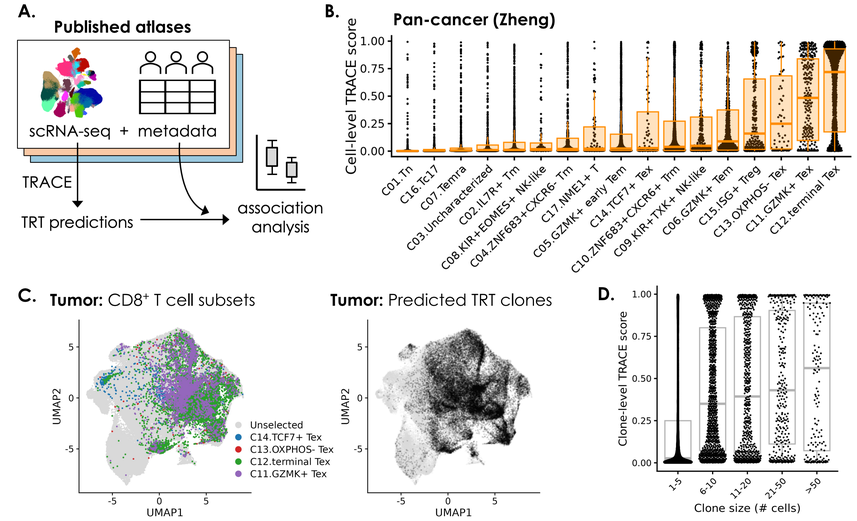


Figure 3. (A) Application of TRACE to tumor infiltrating CD8⁺ T cell scRNA-seq atlases. **(B)** Distributions of TRACE scores across CD8⁺ cells grouped by annotated subtype for the Zheng *et al.* pan-cancer TIL atlas[11]. Only CD8⁺ clusters as defined by the original study are included. **(C)** UMAPs showing location of exhausted CD8⁺ T cells and putative TRT clones predicted by TRACE. **(D)** Distributions of clone-level TRACE scores across clone sizes.

Figure 4: TRACE identifies putative CD8+ TRTs in tumor subtypes but not in inflamed non-tumor and normal tissues

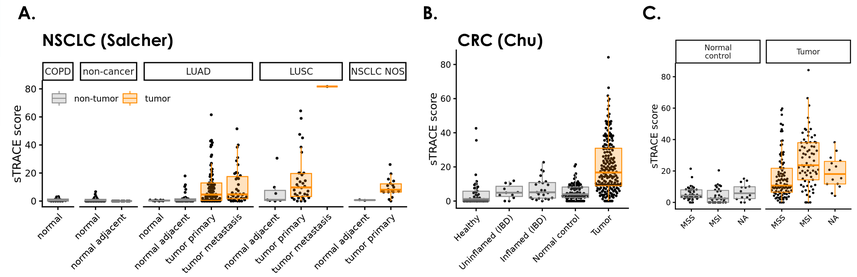


Figure 4. (A) sTRACE scores (fraction of CD8⁺ T cells in sample with TRACE scores ≥ 0.5) for each NSCLC and non-cancerous sample from the Salcher *et al.* lung atlas[12]. NOS, not otherwise specified. **(B)** sTRACE scores for each CRC and non-cancerous sample from the Chu *et al.* CRC atlas[13]. **(C)** sTRACE scores for the CRC and normal control samples from (B) grouped by MSI status. For all panels, tumor sample scores are summarized with orange boxplots and non-tumor scores with gray boxplots.

Conclusions

- TRACE is a tumor reactive CD8⁺ T cell clone-level classifier released with open model weights that can be applied to tissue or blood scRNA-seq datasets
- TRACE exhibited robust performance on held-out TIL and PBMC clones across 50 seeds, achieving a mean F1-score of 0.85 and a mean FPR on PBMCs below 1%
- TRACE predictions were validated by co-culturing *ex vivo* expanded TIL with an autologous melanoma tumor cell line
- Across multiple tumor atlases, TRACE scores were observed to be significantly higher in exhausted CD8⁺ T cells in tumors but not in exhausted cells in normal adjacent or non-cancer samples, suggesting specificity towards identifying tumor-antigen experienced T cells

TRACE preprint bioRxiv

Open Source Code
github.com/KSQtx/TRACE