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Leveraging RNA sequencing for scalable tumor immune repertoire profiling

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INTRODUCTION

Interactions between tumors and the immune system have clinically significant impacts for cancer development and immunotherapy.

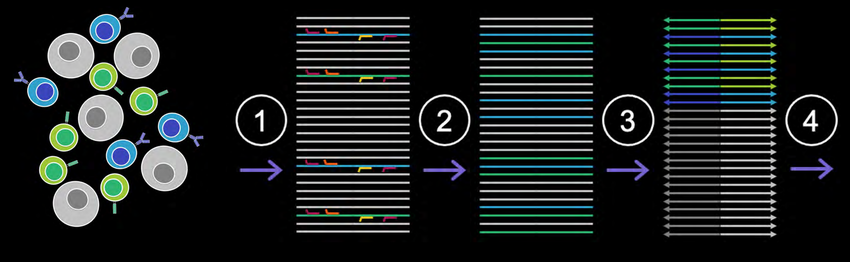
There is an outstanding need to develop methods that accurately profile tumor-immune interactions at scale to maximize the availability of data-driven treatment options for oncology patients.

Immune repertoire sequencing (rep-seq) quantifies hypervariable T-cell receptor (TCR) and B-cell receptor (BCR) sequences to profile relative abundances of tumor-infiltrating lymphocyte clonotypes.

Accumulating evidence indicates that tumor repertoire dynamics (e.g., richness and evenness) may have clinically significant impacts for immunotherapeutic efficacy.

The present study describes the validation of a method that enriches immune repertoire transcripts in a high-volume RNA-sequencing (RNA-seq) workflow to bring rep-seq to oncology patients at an unprecedented scale.

METHODS



1) Tumor sampling
RNA is isolated from formalin-fixed, paraffin-embedded primary tumor samples. Samples harbor a broad spectrum of lymphocyte infiltration, largely dependent on sample tissue origin.

2) TCR/BCR transcript enrichment
Specially designed and optimized hybrid-capture probe pools target genes for seven lymphocyte receptors (TCR-α, TCR-β, TCR-γ, TCR-δ, Ig-Heavy, Ig-κ, and Ig-λ) to enrich immune receptors in RNA-seq output without compromising downstream transcriptomic analysis.

3) RNA-sequencing
Tempus' state of the art RNA-seq platform (tempus.com/genomic-profiling/) provides transcriptomic analysis of tumor samples. TCR/BCR reads enriched by the application of rep-seq probes do not exceed 2% total reads in 95% of RNA-seq runs.

4) Repertoire-sequencing analysis
RNA-seq data is processed using open-source rep-seq software TRUST4 (https://github.com/liulab-dfci/TRUST4). Candidate TCR/BCR reads are aligned against IMGT reference allele sequences and hypervariable complementarity-determining region 3 (*CDR3*) sequence clonotypes. *CDR3* gene assignments and relative abundances are quantified.

STUDY DESIGN

The present study validates sequencing data from 501 primary tumor samples represented below by 14 broad tumor types.

All samples were subjected to the Tempus RNA-seq workflow with the added inclusion of optimized TCR/BCR hybrid capture probe pools.

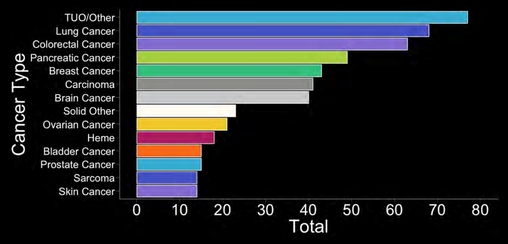


Figure 1. Total number of primary samples by broad cancer type (n=501). TUO = Tumor of Unknown Origin.

RESULTS

Figure 2. Repertoire sizes for 501 primary tumor samples match expected biological trends.

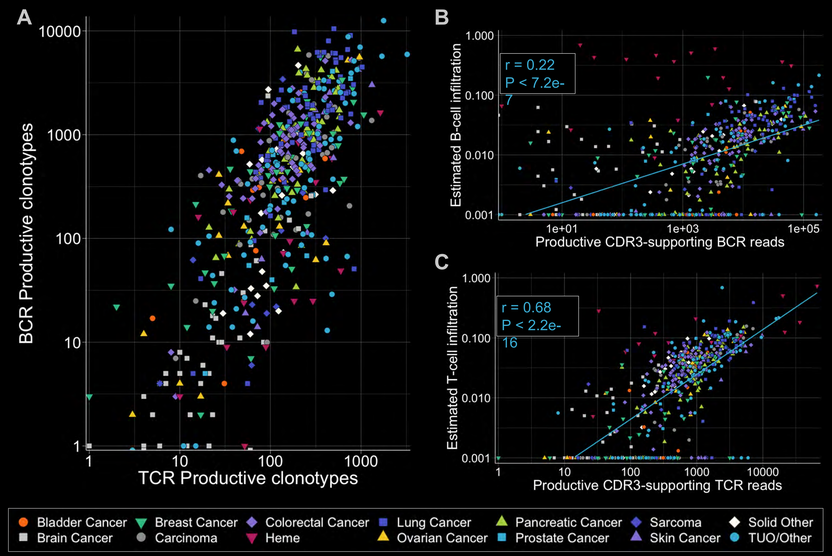


Figure 2. Repertoires generated from 501 tumor transcriptomes demonstrate broad distribution of clonotypic richness. **(A)** Total productive clonotypes (excluding *CDR3* sequences with partial alignments, frameshifts and internal stop codons) for BCRs (Ig-heavy,- κ, and -λ) and TCRs (TCR-α, TCR-β, TCR-γ, and TCR-δ). Gene expression-based estimations (PMID: 30864330) for **(B)** B-cells and **(C)** CD4/CD8 T-cells correlate with clonotype yield (reads supporting productive *CDR3*s) for respective receptors (one-tailed Pearson - 95% CI). Samples with infiltration estimations at or below 0.001 are displayed at that value.

Clusters of tumor tissue types trend with expectations for tissue-specific immune infiltration levels (see lung and brain cancers). Clonotypes mapping to all seven TCRs and BCRs are identified in the majority of samples sequenced, where high background enrichment for Ig light chains elevates BCR yield in large immune repertoires and TCR-γ/-δ clonotypes are detectable yet relatively low in abundance.

Figure 3. TCR-β clonality distributions.

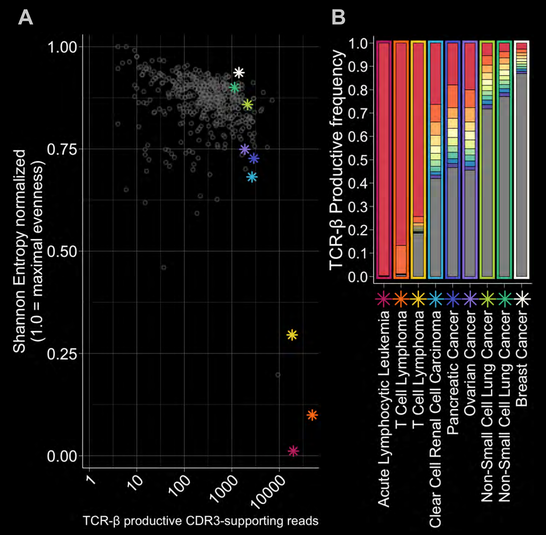


Figure 3. Repertoires demonstrate distributions of clonality/evenness where T-cell- and B-cell-driven cancers demonstrate expected monoclonality. A representative distribution **(A)** of TCR-β repertoire clonality is displayed above. Clonality is quantified via Shannon entropy normalized against the theoretical maximum evenness for a given repertoire size (1 = reads evenly distributed between clonotypes, 0 = monoclonal). Selected samples are highlighted and **(B)** the productive receptor frequency for the top ten clonotypes are displayed (remaining repertoire in grey).

Figure 4. External benchmarking and inter-assay performance.

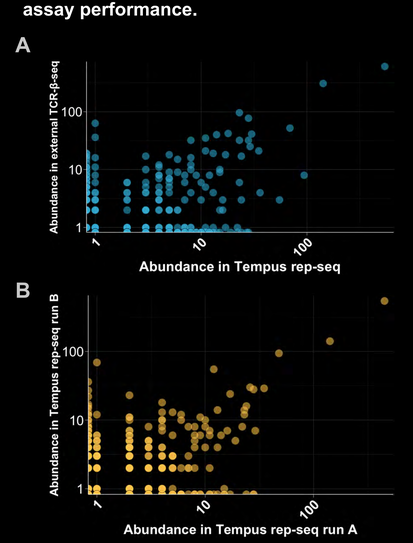


Figure 4. (A) A sample subjected to Tempus enriched RNA-based rep-seq and a highly sensitive external TCR-β receptor DNA sequencing assay. Exact TCR-β *CDR3* nucleotide sequences are quantified and compared between runs in this benchmark and **(B)** separate Tempus rep-seq runs.

While enriched RNA-based rep-seq is naturally less sensitive than stand-alone DNA-based assays, our method detects and recapitulates the relative abundance of the most frequent clonotypes, even in the relatively small TCR-β repertoire displayed above. Consistency is also high for abundant clonotypes in inter-assay tests.

CONCLUSIONS

Enrichment of TCR and BCR transcripts in RNA-seq input via hybrid capture probes is an effective strategy for immune repertoire profiling without compromising transcriptomic analysis.

Repertoire metrics measured in 501 primary tumor samples trend with biological expectations for tissue-specific lymphocyte infiltration and cancer-specific clonality.

Despite being less sensitive than stand-alone assays, this method benchmarks well for detection of the most abundant tumor-localized T-cell clonotypes.

This method allows repertoire profiling to be incorporated into high-volume RNA-seq workflows at scale and is currently being applied to thousands of tumor samples every month at Tempus.

ACKNOWLEDGEMENTS

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