T-cell receptor alpha and beta repertoire profiling using an augmented transcriptome

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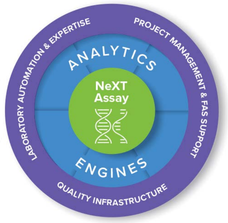
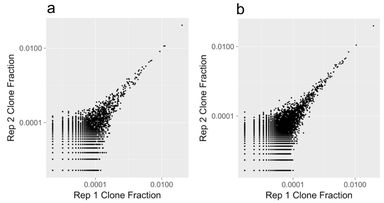
Introduction

The promise of immunotherapy has revealed the need for comprehensive profiling of the tumor and its immune microenvironment. This includes analysis of the T-cell receptor (TCR) repertoire, which has traditionally not been feasible with an exome/transcriptome platform. To address this challenge, we developed ImmunoID NeXT, an augmented, immuno-oncology optimized exome/transcriptome platform designed to provide a more comprehensive view of the tumor and tumor microenvironment (TME) from limited FFPE tumor biopsies. This includes profiling both the TCR α and β chains. We show that ImmunoID NeXT accurately and reproducibly profiles abundant clones, and provides information on the diversity of T-cells in tumor samples.



Accurate immune repertoire sequencing with NeXT



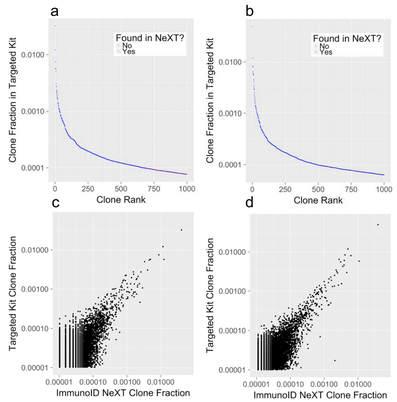
Abundances of clones found in common between separate preps of a healthy PBMC sample. R^2 values of 0.99 for both TCRα (a) and TCRβ (b).

Reproducible profiling of TCRα and TCRβ

We first evaluate the reproducibility of ImmunoID NeXT at profiling both TCRα and TCRβ from RNA by analyzing the concordance of clones using replicates of PBMCs. Abundances of clones shared between replicates have a very high concordance, showing that even with a diverse repertoire in healthy PBMCs, our TCR profiling provides reproducible results.

Accurate profiling of top clones

Next, we compare the concordance of clones from ImmunoID NeXT to the top clones from a standalone TCR sequencing approach. Compared to the standalone approach, we identify 96% of the top 1000 TCRα clones, and 99% of the top 1000 TCRβ clones, both with highly concordance abundances across all shared clones (R^2=0.95 and R^2=0.94 in TCRα and TCRβ, respectively). This shows that our approach has the capability to accurately profile top clones.

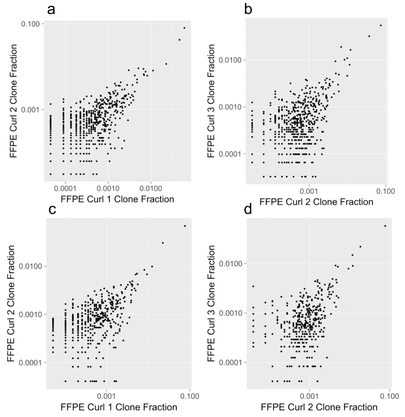


Identification of the top 1000 clones as identified in a targeted kit for TCRα (a) and TCRβ (b).
Comparison of the abundances of clones found in both the targeted kit vs. ImmunoID NeXT (clones ≥0.00001) for TCRα (c) and TCRβ (d).

Repertoire profiling with tumor FFPE samples

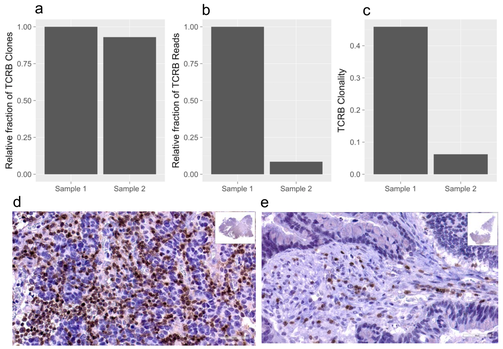
Reproducibility in FFPE samples

We also analyze patient-derived colorectal cancer (CRC) FFPE tumors to characterize the profiles of tumor-infiltrating immune repertoires. First, we analyze the reproducibility of clones identified using serial sections of FFPE samples. We observe a strong concordance of the abundances for shared clones between the sections, showing that our approach is robust to degraded FFPE samples.



Comparison of abundances for clones found in common between serial sections of a tumor FFPE sample. R^2 values of 0.92 (a) and 0.89 (b) for TCRα, and 0.94 (c) and 0.91 (d) for TCRβ.

Clonal distributions compared to immunostaining



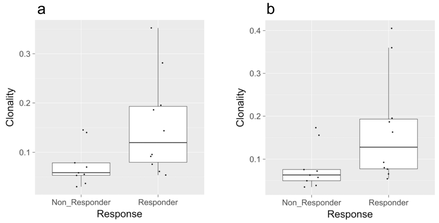
(a,b) Comparison of Sample 2 relative to Sample 1 of fraction of TCRβ clones and of TCRβ reads. (c) TCRβ clonality calculated for Samples 1 and 2. IHC of CD3 (brown) for Samples 1 (d) and 2 (e).

We compare TCRβ profiling with immunohistochemical (IHC) staining of CD3+ cells in two additional CRC tumor tissues. In our analysis of T-cell infiltration, Sample 1 has only a slightly higher number of clones than Sample 2, but a much higher number of TCRβ reads and clonality than Sample 2. This is reflected in the IHC data, where Sample 1 has significant infiltration of T-cells (49%) compared to Sample 2 (23%). This shows how different metrics of TCR profiling provide complementary biological information.

Profiling of TCR clonality in tumor samples

Clonality in melanoma patients

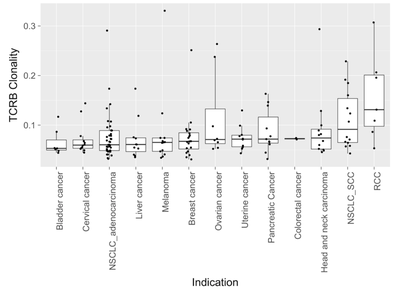
To further analyze the utility of TCR clonality, we use ImmunoID NeXT to profile pre-treatment tumor samples in a cohort of melanoma patients who underwent PD-1 blockade therapy. In this cohort, clonality is significantly different in responders to checkpoint inhibition based on either TCRα (p=0.028) or TCRβ (p=0.022).



Comparison of clonality for responders and non-responders of checkpoint blockade therapy for both TCRα (a) and TCRβ (b).

Diversity of clonality across tumor indications

Finally, we use ImmunoID NeXT to profile the diversity of TCRβ clonality across 168 solid tumor samples. This ongoing effort to profile a diverse set of indications can provide us with a deeper understanding of the distributions of immunological metrics, such as TCR clonality, across samples.



Conclusion

The ImmunoID NeXT platform can provide insight into the diversity of the immune repertoire, highlighting one aspect of the platform's ability to provide analysis of the TME. We demonstrate that ImmunoID NeXT is reproducible and accurate at profiling TCRα and TCRβ clones, as well as robust to degraded FFPE samples. We also describe how profiling clonal distributions using ImmunoID NeXT can be used to gain understanding of the immunological composition of the TME. Finally, we show how ImmunoID NeXT can profile the diversity of the TCR repertoire in tumor samples. In summary, by combining exome/transcriptome sequencing with TCR characterization into a single assay, our ImmunoID NeXT platform enables comprehensive immuno-genomics characterization of a tumor sample while reducing overall sample requirements and cost.

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