B-cell receptor heavy chain repertoire profiling using an augmented transcriptome

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Introduction

Comprehensively profiling the tumor and tumor microenvironment (TME) can help provide a more complete view of the complex interactions between the tumor and immune system, potentially furthering our understanding of tumor progression and response to treatment. We have developed an augmented, immuno-oncology-optimized exome/transcriptome platform, ImmunoID NeXT™, which provides a comprehensive view of the tumor and TME from limited FFPE tumor biopsies. Studies have indicated that tumor-infiltrating B cells can have both pro- and anti-tumor effects depending on their phenotypes, and that characterization of the B cell infiltrate can be a prognostic factor[1]. In addition to previous work demonstrating the ability to profile the T-cell receptor (TCR) α and β chains, we have now added the ability to profile the B-cell receptor (BCR) heavy chain (IGH). We show here that ImmunoID NeXT is able to accurately and reproducibly profile abundant B-cell clones, as well as provide information on the diversity of B-cells in tumor samples.

Tumor and immune profiling with the ImmunoID NeXT platform

To address the challenge of providing characterization of both the tumor and TME, we have developed the ImmunoID NeXT Platform™, an augmented, immuno-oncology-optimized exome/transcriptome platform designed to provide comprehensive information from a single FFPE tumor sample.

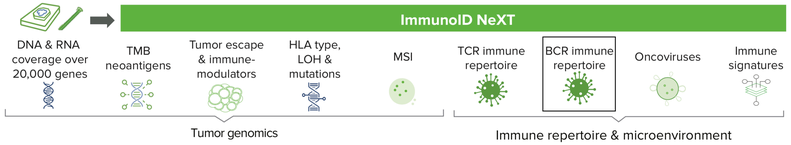


Figure 1: overview of results provided in ImmunoID NeXT.

Accurate BCR sequencing with ImmunoID NeXT

Reproducible profiling of IgG, IgM, and IgA

We first assessed the reproducibility of ImmunoID NeXT by profiling IGH clones in replicates of a PBMC sample. Abundances of clones found across replicates for the major isotypes (IgG, IgM, and IgA) were highly concordant. This demonstrates that in a sample with a diverse repertoire our BCR profiling provides reproducible results.

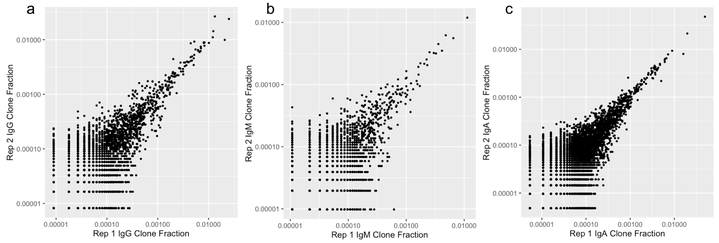
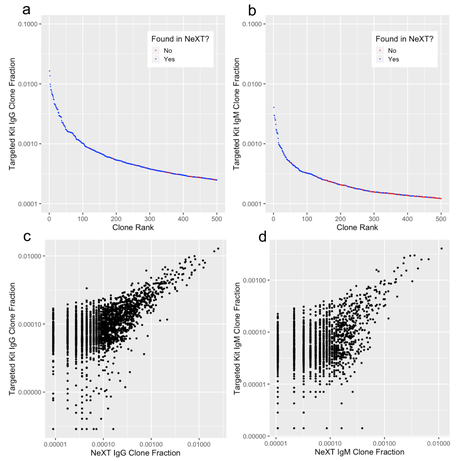


Figure 2: abundances of clones in replicates of a healthy PBMC sample. R^2 values of 0.86 for IgG (a), 0.92 for IgM (b), and 0.97 for IgA (c).

Accurate profiling of top clones

Next, we compared the concordance of clones identified using ImmunoID NeXT to those found using a standalone BCR profiling approach, which specifically targets IgG and IgM. When comparing to the standalone BCR sequencing method, we identify 475 of the top 500 clones in IgG, and 387 of the top 500 in IgM. We also observe highly concordant abundances. This shows that our BCR results are able to accurately profile high-abundance clones.

Figure 3: identification of top clones compared to a targeted kit for IgG (a) and IgM (b). Abundances for clones found in both the targeted kit and ImmunoID NeXT, with R^2 values of 0.82 for IgG (c) and 0.72 for IgM (d).



Unbiased profiling of all isotypes

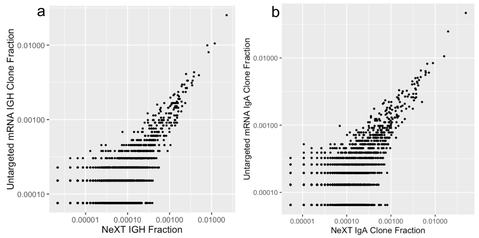


Figure 4: abundances of clones found in both the untargeted RNA-seq method and ImmunoID NeXT for all isotypes (a) and specifically IgA (b).

We also assessed the concordance of results using ImmunoID NeXT to untargeted RNA-seq, which can provide a shallow but unbiased estimate of clonotype abundances for all isotypes. First, we observe highly concordant abundances across all IGH clones (R^2=0.95). When we specifically compare IgA clones, which are profiled by ImmunoID NeXT and not covered by the targeted method, we still observe highly concordant abundances (R^2=0.95). This suggests that ImmunoID NeXT is able to provide an unbiased view of all high-abundance IGH clones.

References

1. Sharonov, George V et al. "B cells, plasma cells and antibody repertoires in the tumour microenvironment." *Nature reviews. Immunology* vol. 20,5 (2020): 294-307. doi:10.1038/s41577-019-0257-x

BCR profiling with tumor FFPE samples

Reproducibility in FFPE samples

We also characterized the profiles of tumor-infiltrating BCR repertoires using a patient-derived FFPE tumor sample. When comparing subsequent curls of the tumor sample, we achieve a high concordance of clonal abundances. This demonstrates that our approach is robust to degraded FFPE samples.

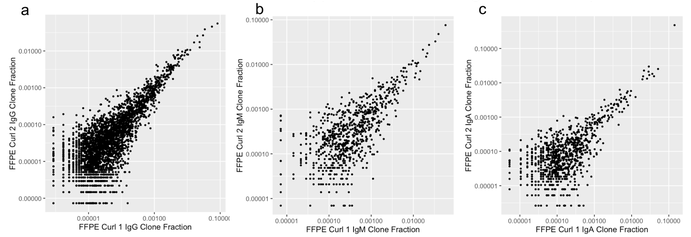


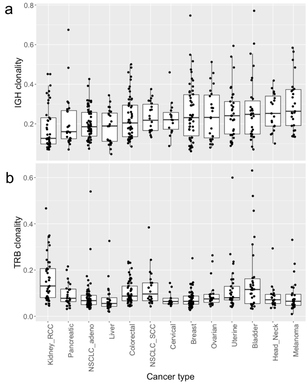
Figure 5: abundances for clones in serial sections of a tumor FFPE sample. R^2 values of 0.93 for IgG (a), 0.92 for IgM (b), and 0.76 for IgA (c).

Profiling BCR clonality across tumor samples

Diversity of clonality across tumor types

Finally, we use ImmunoID NeXT to profile the diversity of immune receptor clonality across 423 solid tumor samples. This is part of an ongoing effort to profile a diverse set of tumor types, which can further our understanding of the distribution of immune-related metrics. With the addition of IGH clonality to our previously-shown TRB clonality, we demonstrate how the comprehensive profiling in ImmunoID NeXT can provide a more complete view of the immune composition.

Figure 6: distribution of clonality of IGH (a) and TRB (b) across cancer types.



Conclusion

The ImmunoID NeXT platform is able to provide insight into the diversity of the BCR repertoire, adding to the platform's ability to profile the TME. We demonstrate that ImmunoID NeXT is able to accurately and reproducibly profile high-abundance BCR heavy chain clones, including coverage of all major isotypes. In addition, we show how ImmunoID NeXT can profile the diversity of the BCR repertoire across a variety of tumor samples. Combined with the platform's TCR profiling capabilities, ImmunoID NeXT can provide insight into the diversity of the immune repertoire, contributing to its ability to provide comprehensive analysis of both the tumor and TME from a single FFPE sample.

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