TRANSCRIPTOME AUGMENTATION PROVIDES ACCURATE AND SENSITIVE QUANTIFICATION OF GENES ASSOCIATED WITH THE TUMOR MICROENVIRONMENT


Background Tumors harbor a complex and dynamic ecosystem of malignant, immune, and stromal cells. While malignant cells dictate much of the tumor biology, there is evidence that the tumor microenvironment (TME) also plays a significant role in disease progression and response to therapy. The role of the immune cells is particularly relevant in immunotherapy, and multiple transcriptome-based biomarkers have shown utility in predicting the efficacy of immune checkpoint blockade. However, little is known about the benefits of enhancing the depth and uniformity of transcriptome sequencing coverage for quantifying the TME cell type composition.

Methods We have developed the ImmunoID NeXT Platform®, which combines high-quality exome and transcriptome sequencing with advanced informatics designed for immune-oncology to comprehensively characterize the tumor and TME from a single FFPE tumor sample. Proprietary augmentation technology was applied to bolster sequencing depth in regions of low coverage across approximately 20,000 genes, enhancing transcriptome coverage uniformity. We processed and sequenced 32 PBMC samples, in-vitro cell mixtures (CD8, CD4, Tregs, B-cells), and over 100 purified cell types to assess the biases and performance of gene expression quantification using the augmented transcriptome. Immune cell composition was validated using flow cytometry. Using purified cell types, we applied differential expression analysis to identify genes preferentially expressed in target cell types. Finally, we confirmed the augmented transcriptome identifies well-established cell-type marker genes and novel cell-type enrichment genes fit for deconvolution.

Results We observed that the ImmunoID NeXT Platform benefits read coverage and uniformity for the majority of genes as compared to both PBMC (PolyA+) and tumor samples (rRNA-depletion). We identified genes preferentially expressed in immune, stromal and granulocyte cell types, showing high overlap with previous literature, and we describe over 1,000 new potential markers fit to assess cell type enrichment in reference samples. To demonstrate that coverage augmentation did not introduce biases disrupting the collinearity between cell fractions and gene expression, we profiled in-vitro cell mixtures. We found that marker genes for Tregs, CD4, CD8, and B-cells are linearly correlated with the fraction of cells mixed and verified by flow cytometry. For instance, well-established CD8 markers show a strong correlation between cell fraction and expression (CD8A corr=0.947 p-value=2.55e-12).

Conclusions We show that ImmunoID NeXT® accurately captures and augments the transcriptome of PBMC and FFPE samples. Applying augmented transcriptome coverage to the assessment of the TME benefited the identification of marker genes for cell type enrichment analysis without introducing bias.