# Illuminating the Transcriptome: Design and Technical Performance of Whole Transcriptome Atlas for GeoMx Digital Spatial Profiler

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## Abstract

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- High-plex spatial technologies are needed to profile gene expression while maintaining information on tissue architecture
- The Human Whole Transcriptome Atlas (HuWTA) for GeoMx<sup>®</sup> DSP uses a curated approach to target 99.5% of protein-coding genes while optimizing readout efficiency
- Here, we demonstrate the capabilities of HuWTA by benchmarking against other bulk and spatial technologies
- We also examined unique morphological structures in multiple tissues to outline guidelines for use of HuWTA.
- Finally, we used these guidelines in experiments profiling directed AOIs across healthy and diseased kidney samples
- Our results demonstrate that HuWTA successfully integrates transcriptome-scale spatial biology with sophisticated AOI design to enable flexible discovery with spatial context.





A. Table describing content of WTA. 10 highest expressing genes, as well as mitochondrial genes, were dropped from the design to optimize sequencing efficiency B. Sequencing space example to illustrate that HuWTA content allows for a greater percentage of reads to be spent on targets of interest, as opposed to high-expressing but biologically uninformative genes. C. Illustration of comprehensive probe coverage across the transcriptome.

#### HuWTA demonstrates high sensitivity, and concordance with RNAseq

Absolute transcript number sensitivity for HuWTA



**Concordance with RNAseg and RNAscope** Scatterplots for a representative cell line (faceted by AOI size) across cell lines Ė 0.7 0 2 4 -2 4 -2 50 log10(RNAseg TPM) AOI size (um) ≤ 0.5-

-2 -1

-1 0



Spearman's correlations







**Biology-driven AOI selection** 

A. Colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) were annotated by a pathologist and ROIs were placed in different regions: Tumor, Hyperproliferative, and Invasive margin. ROIs were segmented into Tumor and Immune. B. (left) Total number of genes detected by percent AOIs in which that gene was detected. (right) Number of genes detected per AOI in different segment types.

#### **Targeted AOIs capture relevant biology**





0.2 -

0.1

E 0.0 -

0.10

0.05

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# Technical benchmarking in tissue: subsampling to determine ideal read depth

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Subsampling AOIs decreases their percent unique













**C**. 212 AOIs originally sequenced above 300 raw reads/um<sup>2</sup> were randomly subset with 5 replicates at each read depth. As read depth decreases, percent unique increases (points = average, error bars = 1 st dev) **D.** Description of AOI size bins



E. Primary and secondary analysis spearman correlations between subsets and results at 300 raw reads/ $\mu$ m<sup>2</sup> for each AOI. Gene counts indicate number of genes above LOQ. Q3 counts normalized by dividing the 3rd quartile for each AOI by the geomean of the 3rd quartiles in the study. Decon values from SpatialDecon package. Single sample GSEA (ssGSEA) pathway scores for each AOI. 100 raw reads/ $\mu$ m<sup>2</sup> called out with vertical line to show analyses do not improve with more sequencing.

### Discovering spatial biology in the kidney: HuWTA uncovers heterogeneity in health and disease

0.50 increase in immune cells in DKD. These changes are 0.25 more severe in diseased glomeruli that are more morphologically abnormal. Box plots (left) show the fraction of each cell type in all healthy and diseased glomeruli separated by pathological annotation. Bar plots (right) show fraction of each cell type in three example glomeruli from a disease sample, one that is healthy (left) and two that are abnormal (right).



## Conclusions

- Curated HuWTA content covers 99.5% of proteincoding genes
- HuWTA is concordant with other platforms
- HuWTA can detect transcripts present at 1 copy per cell
- HuWTA measures the whole transcriptome in a single AOI
- HuWTA results are optimized with 100 sequencing reads/ $\mu$ m<sup>2</sup>
- Flexible AOI selection captures gene expression differences in biological structures in healthy and diseased kidney
- Cell type deconvolution reveals spatial heterogeneity across diseased samples



