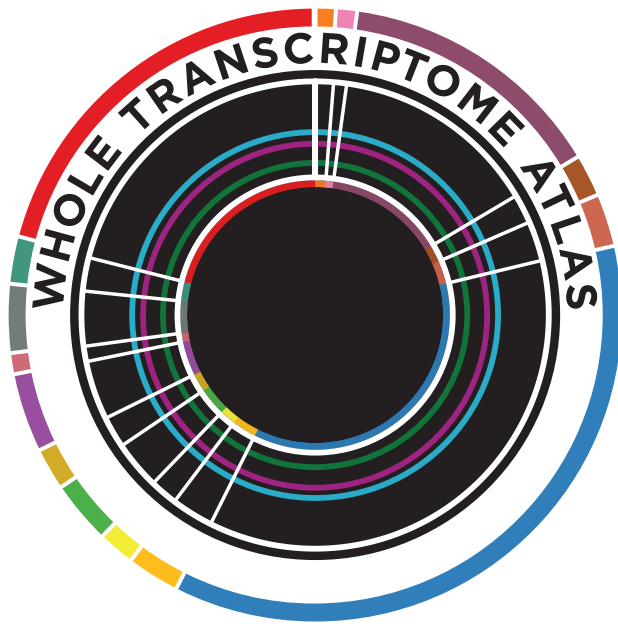


GeoMx® Whole Transcriptome Atlas

The GeoMx Digital Spatial Profiler with the GeoMx Whole Transcriptome Atlas (WTA) empowers researchers to explore biology across a tissue. WTA is compatible with common sample types such as formalin-fixed paraffin embedded (FFPE) or fresh frozen (FF) and across all human tissues. The human whole transcriptome is measured in each region of interest (ROI) to uncover biological changes at specific locations in the tissue.



Product Highlights

- Whole transcriptome coverage with probes specific to protein coding mRNA sequences
- Superior sensitivity to detect 1000s unique genes in <50 µm regions
- Robust performance across sample types including FFPE with high concordance with RNA-seq and RNAscope™
- Map single cell RNA-seq populations to their tissue location
- Integrated Illumina library prep protocols and streamlined NGS analysis pipelines
- Flexibility to customize with up to 60 additional targets to assay non-coding RNA, synthetic DNA or exogenous genes
- Visualization and statistical analysis using GeoMx Data Suite and bioinformatics toolset

Discover a new way to look at biology

The GeoMx Whole Transcriptome Atlas delivers the maximum amount of sensitivity and confidence in each transcript through its unique probe architecture. WTA contains over 18,000 protein-coding genes based on the human gene nomenclature committee (HUGO¹) database cross-referenced with available mRNA sequences in the National Center for Biotechnology's Information (NCBI) RefSeq database² (Figure 1). Along with a high specificity for their target molecules, DSP RNA probes are screened for a myriad of factors including melting temperature, GC content, off target hits, and repetitive sequences. Each probe is assigned a unique DSP barcode for downstream molecular counting on an Illumina sequencer. The targeted nature of WTA avoids the many pitfalls of poly(A) pulldown by delivering increased sensitivity even in heavily degraded tissues such as FFPE. With GeoMx WTA, researchers can explore pathways across the whole transcriptome in user defined regions of interest.

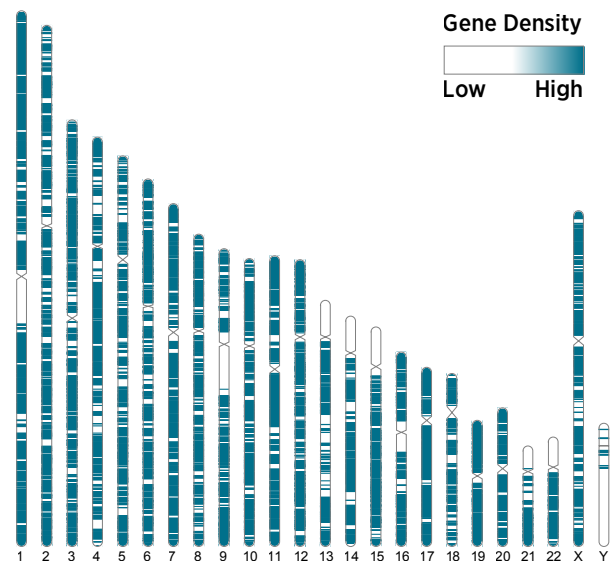


Figure 1: Karyogram illustrating the density of genes in the human genome. The transcripts from these genes make up the human transcriptome and are measured by GeoMx WTA.

Explore the biology that matters

The GeoMx WTA offers high sensitivity and specificity with thousands of genes detectable across region of interest (ROI) of various sizes. In a sensitivity analysis in FFPE with cell pellet arrays, colorectal carcinoma CRC and non-small cell lung cancer NSCLC, over 5000 genes were detected above the limit of quantification.

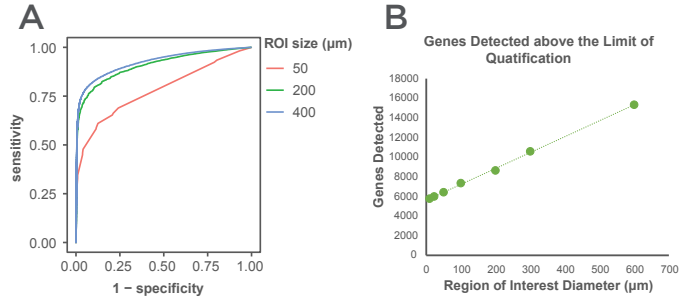


Figure 2: A) Using RNA-seq transcripts per million as the ground truth for the expression of each gene, the ROC curve shows high sensitivity and specificity. B) GeoMx WTA detects a large number of genes across many regions of various sizes.

Spatial RNA profiling aligns with RNASeq and RNAscope

Traditional gene expression technologies are unable to capture heterogeneity of the transcriptome with spatial context. Bulk RNA sequencing and single cell RNAseq have delivered interesting clinical and cell type signatures but localizing these signatures has proven challenges with protein based IHC. Other, more precise, technologies like fluorescently-labeled *in situ* hybridization lack the plex to truly make new discoveries. WTA delivers both spatial precision and high plex. Furthermore, WTA compares favorably RNA sequencing and RNAscope (Figure 3) and is able to confidently detect transcripts in small regions.

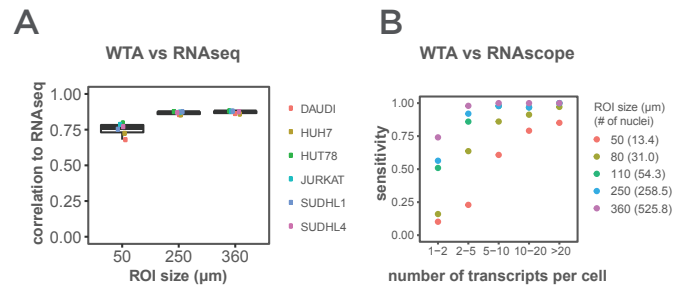


Figure 3: A) GeoMx Human WTA performance compared to RNA-seq with 3 ROI sizes run on formalin-fixed paraffin embedded (FFPE) cell pellet arrays. B) Comparing RNAscope and WTA counts illustrates the high sensitivity of WTA even with small ROI sizes.

Map the location of your cell populations

Spatial analysis of a non-small cell carcinoma's sample using the GeoMx SpatialDecon algorithm³ reveals cell type abundance in each region of interest (ROI) (Figure 4). Researchers can use the pre-defined cell definitions or their single cell RNAseq data to assess cell type abundance throughout the tissue.

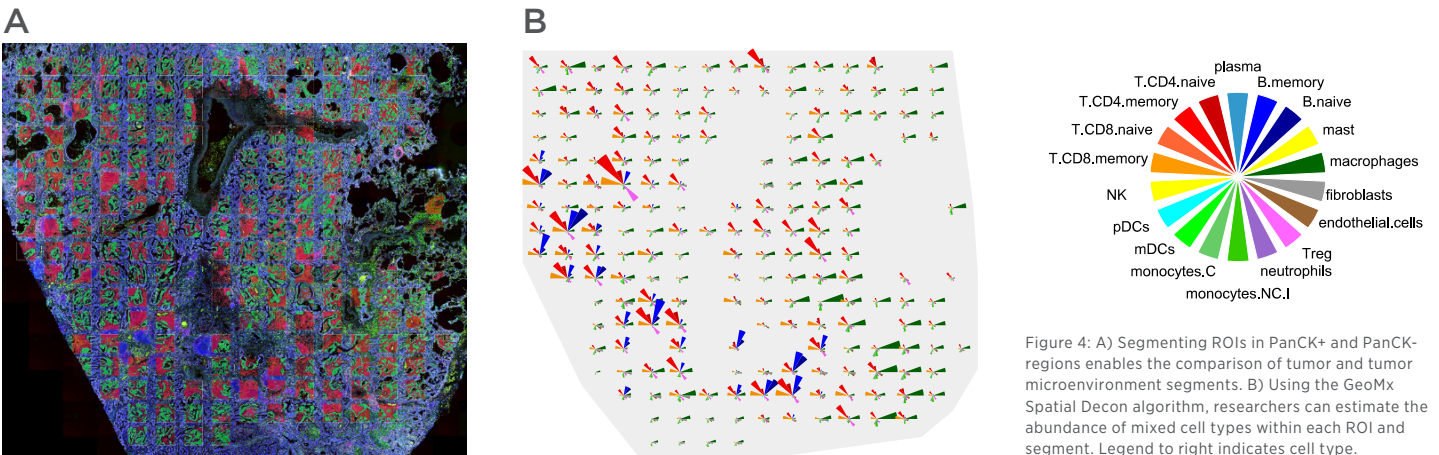
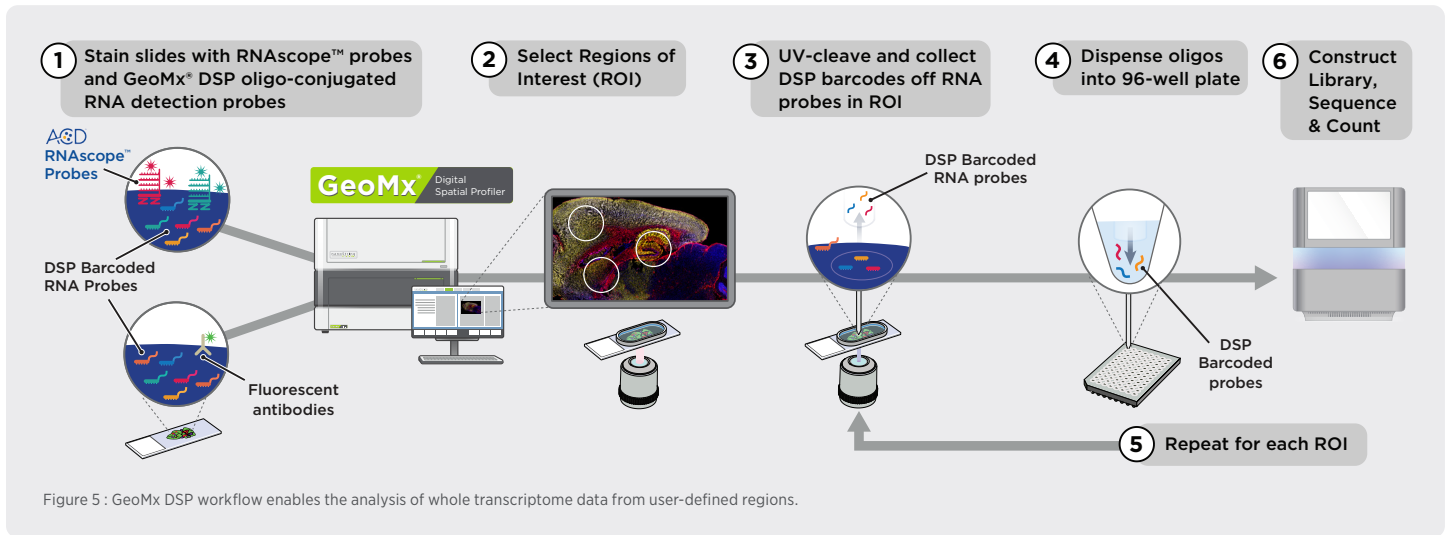


Figure 4: A) Segmenting ROIs in PanCK+ and PanCK- regions enables the comparison of tumor and tumor microenvironment segments. B) Using the GeoMx Spatial Decon algorithm, researchers can estimate the abundance of mixed cell types within each ROI and segment. Legend to right indicates cell type.

GeoMx WTA Workflow

GeoMx RNA assays contain *in situ* hybridization (ISH) probes conjugated to unique DNA indexing-oligonucleotides (DSP barcodes) via a UV-photocleavable linker. After selecting regions of interest (ROIs) on the GeoMx DSP, the DSP barcodes are UV cleaved and collected (Figure 5). During library preparation, the DSP barcodes are tagged with their ROI location then sequenced on an Illumina sequencer. DNA oligonucleotide sequences contain ROI indices mapping them back to their tissue location, an RNA target identification sequence matching them to their ISH probes, and a unique molecular identifier (UMI) to deduplicate reads. Sequenced oligonucleotides are processed then imported back into the GeoMx DSP platform for integration with the slide images and ROI selections for spatially-resolved RNA expression.



GeoMx® Data Analysis

GeoMx software uniquely combines whole tissue visualization at single cell resolution with advanced ROI selection to enable comprehensive spatial profiling of tissue sections. The fully integrated workflow tracks image data to corresponding profiling data, allowing users to easily go from data collection to data analysis and to interact with either data type in real time. The data analysis suite provides multiple options to assess the quality of the raw data and various methods to normalize data sets. Moreover, multiple analysis methods are available, including statistical methods, and cluster and pathway analysis. Multiple data visualizations are available to enable data exploration and are then exportable as publication-quality figures. Visualization plots include: heatmap, dendograms, bar graph, box plot, scatter plot, line/trend plot, volcano plot, forest plots, and 3-D PCA. Analysis and visualization can be extended through the integration of R-scripts into the Data Analysis Suite which are available on GeoScript Hub, or through export of the raw or normalized data to external tools including our GeoMxTools R package available in Bioconductor™.

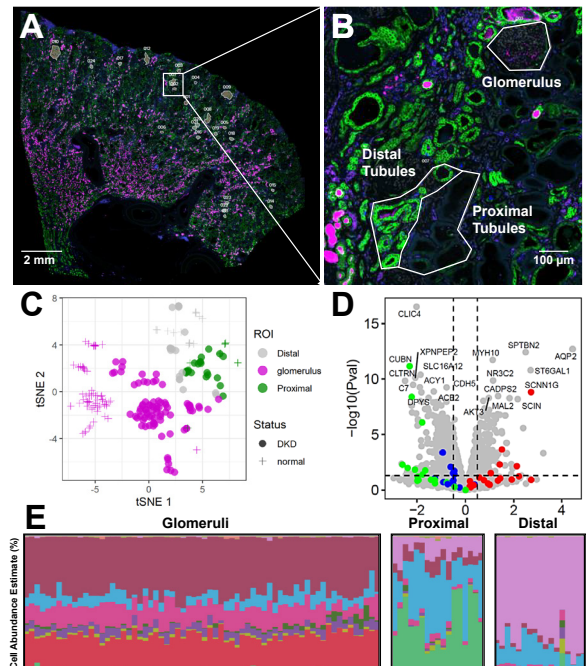


Figure 6: A-B) ROI selection strategy in kidney to study normal and diseased (DKD) kidney C) tSNE representation of glomerulus (magenta), proximal (green) and distal (gray) tubules D) Differential expression showing genes from scRNA Seq expressed in podocytes (blue), proximal (green) or distal (red) tubules. E) Cell type abundance estimates based on scRNA Seq from kidney samples.

Ordering Information

GeoMx Whole Transcriptome Atlas			
Product	Product Description	Quantity	Catalog Number
GeoMx Human Whole Transcriptome Atlas Human RNA for Illumina Systems	RNA Probe set that targets 18,000+ transcripts for human protein coding genes plus ERCC negative controls to profile the whole transcriptome. Excludes uninformative high expressing targets such as ribosomal subunits. Includes RNA probes designed for Illumina NGS readout with the Seq Code library prep.	4 Slides	GMX-RNA-NGS-HuWTA-4
GeoMx Mouse Whole Transcriptome Atlas <i>Mouse RNA for Illumina Systems</i> (Available for Pre-order)	RNA Probe set that targets 21,000+ transcripts for mouse protein coding genes plus ERCC negative controls to profile the whole transcriptome. Excludes uninformative high expressing targets such as ribosomal subunits. Includes RNA probes designed for Illumina NGS readout with the Seq Code library prep.	4 Slides	GMX-RNA-NGS-MsWTA-4
GeoMx Morphology Kits			
Product	Product Description	Quantity	Catalog Number
GeoMx Solid Tumor TME Morphology Kit <i>Human RNA Compatible</i>	Morphology kit for visualization of human solid tumors and the tumor microenvironment. For use with RNA assays. Includes fluorescent antibodies against Pan-CK, CD45, and a nuclear stain.	12 slides	GMX-RNA-MORPH-HST-12
GeoMx Melanoma TME Morphology Kit <i>Human RNA Compatible</i>	Morphology kit for visualization of human melanoma and the tumor microenvironment. For use with RNA assays. Includes fluorescent antibodies against S100B/Pmel17, CD45, and a nuclear stain.	12 slides	GMX-RNA-MORPH-HMEL-12
Additional Assay Reagents			
Product	Product Description	Quantity	Catalog Number
GeoMx Seq Code Pack <i>Compatible with Illumina Systems</i>	NGS readout reagents for GeoMx DSP RNA and protein analysis. Includes two Seq Code primer plates (choice of A&B, C&D, E&F, or G&H) and two universal enzyme master mixes.	192 AOI	GMX-NGS-SEQ-[XX]
GeoMx RNA Slide Prep Kit for FFPE	Sample prep reagents for GeoMx DSP RNA analysis. Includes Buffer W, Buffer S, and Buffer R.	12 slides	GMX-PREP-RNA-FFPE-12
GeoMx DSP Collection Plate	Barcoded collection plates for use on the GeoMx DSP. Required for AOI tracking. Kit includes 12 plates covering 1,152 AOI.	1 Pack	GMX-DSP-COLL-PLT
GeoMx DSP Instrument Buffer Kit	Buffer kit for the GeoMx DSP. Includes Buffer S and Buffer H. Sufficient for ~48 samples with ~18 AOI each. Volume requirements may vary based on experimental design.	1 Kit	GMX-DSP-BUFF-KIT

Selected Panel References

1. HUGO Gene Nomenclature Committee. <https://www.genenames.org/>
2. RefSeq: NCBI Reference Sequence Database. <https://www.ncbi.nlm.nih.gov/refseq/>
3. Danaher, et al. Advances in mixed cell deconvolution enable quantification of cell types in spatially-resolved gene expression data. bioRxiv pre-print.

For more information, visit nanosttring.com/GeoMx-WTA

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