Spatially-resolved *In Situ* Expression Profiling using the GeoMx® Cancer Transcriptome Atlas Panel in FFPE tissue

NanoString Technologies 530 Fairview Avenue North, Seattle, WA 98109

Summary

The emerging field of spatial genomics represents a significant advance for biology. To drive new discoveries in spatial genomics and immuno-oncology, we introduce the GeoMx® Cancer Transcriptome Atlas (CTA) Panel for comprehensive spatial analysis of cancer pathways using the Nanostring GeoMx Digital Spatial Profiler (DSP). We demonstrate profiling of 1800+ immuno-oncology targets in the tumor, microenvironment, and immune compartments of archival FFPE tissue sections, coupled to downstream Next Generation Sequencing (NGS) readout to enable high-throughput workflows.

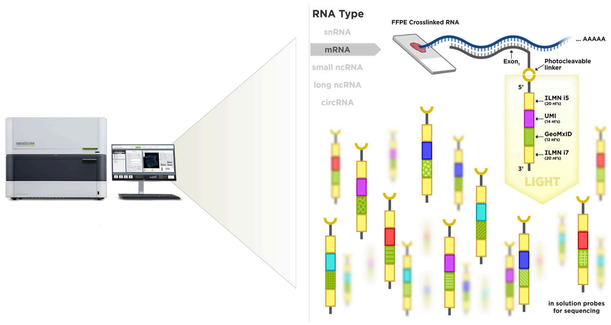
1. High-plex spatial RNA molecular profiling with GeoMx® CTA was performed as follows:
2. Photocleavable DNA oligonucleotides tags were coupled to 8000+ *in situ* hybridization probes targeting 1800+ genes. These reagents were allowed to bind targets directly on slide-mounted FFPE tissue sections.
3. ROIs were identified and selected using GeoMx DSP, and ROI-specific oligonucleotide tags were released using ultraviolet exposure.
4. Released oligonucleotide tags from each ROI were collected and deposited into designated wells on a microtiter plate, allowing well indexing of each ROI during NGS library preparation.
5. After indexing, the entire plate was pooled into a single tube for purification and then sequenced on an Illumina instrument.
6. NGS reads were processed into digital counts and mapped back to each ROI, generating a map of transcript activity within the tissue architecture.

We compared data from experiments in which the GeoMx CTA Panel and bulk RNA-seq were performed on the same samples. Overall, we found good correlation between pseudo-bulk GeoMx CTA (sum of ROIs) and RNA-seq from the same tissue specimen. Individually, however, each ROI showed a distinct expression pattern from bulk, and ROI expression patterns clustered based on similar tissue morphology. Importantly, GeoMx CTA was able to detect a higher number of genes with low expression within the microenvironment and immune spatial compartment compared to bulk RNA-seq, providing a detailed look at the anti-tumor immune response. These data demonstrate that GeoMx offers high sensitivity for genome-scale expression profiling while preserving critical information about tissue architecture.

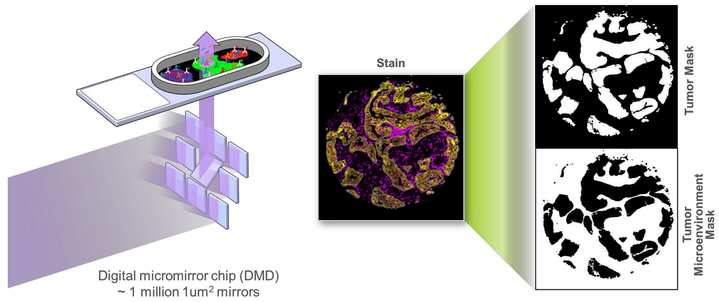
GeoMx® DSP technology is for Research Use Only and not for use in diagnostic procedures.

Introduction

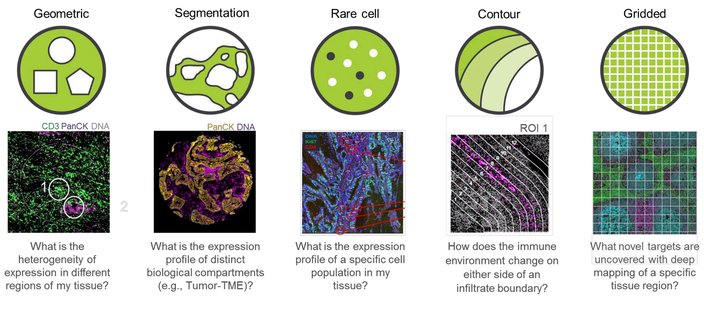
GeoMx® DSP High-plex RNA Chemistry: Sequencing of Photocleaved Barcodes



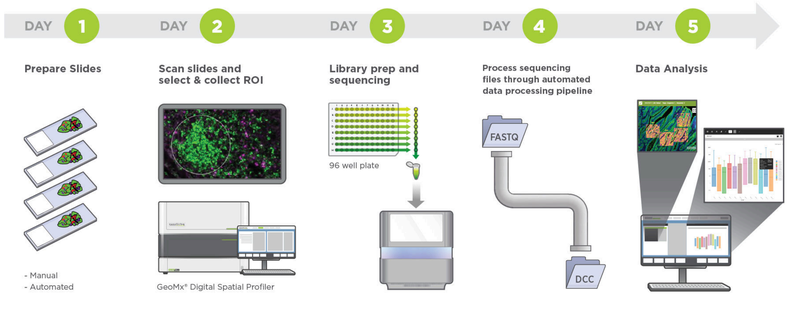
DSP Adaptive Optics Auto-Configure to Region of Any Shape or Size



DSP Adaptive Optics Enables Flexible Profiling Strategy



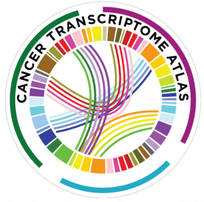
GeoMx® DSP NGS workflow



Results

Cancer Transcriptome Atlas (CTA) : Basic Discovery to Translational Studies

Curated & validated content for cancer research

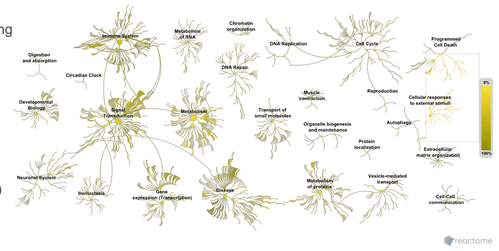


Technology Access Program (TAP) Open Now

- **Expansive content:** 1,833 genes across 55 pathways
- **Comprehensive biology:** Tumor biology, immune response, microenvironment, low expresssors (Cytokines, chemokines, transcription factors etc)
- **Curated annotations:** Includes popular nCounter gene expression panels and signatures (PAM50, TIS)
- **Customization:** Ability to add genes of interest
- **Minimal sequencing:** 30-50M reads/sample

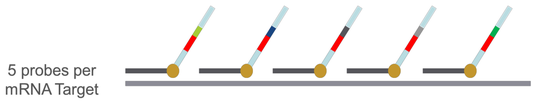
Cancer Transcriptome Atlas Sources

- **Integrated nCounter Panels**
  - PanCancer Immune Profiling
  - PanCancer Pathways
  - PanCancer IO 360
  - Metabolic Pathways
- **Scientific Community input**
- **Annotated Gene Sets**
  - Immune Cell types
  - Tumor Inflammation
  - Tumor Signatures (PAM50)
  - 95 Biology Pathways

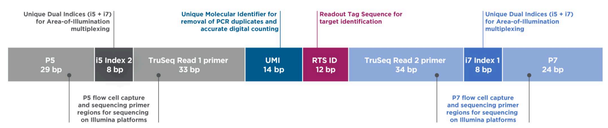


CTA Targeted Panel is Designed and Validated for High Performance

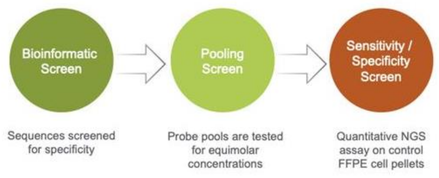
- CTA panel contains 8000+ probes with five probes per mRNA target.



- CTA library construct is designed for standard Illumina sequencing workflows.

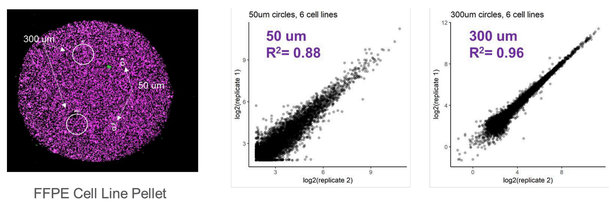


- CTA pooled probes proceed through a standard validation process.

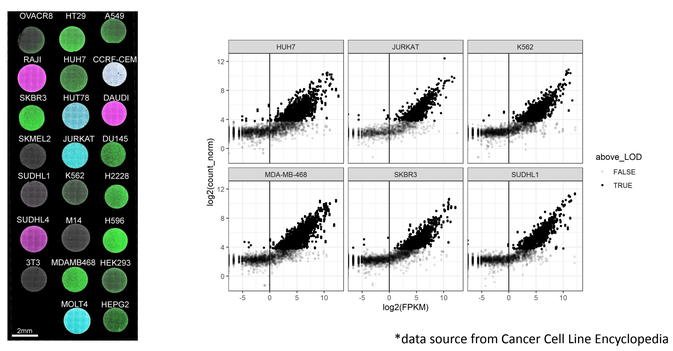


CTA Gene Expression Profiles are Reproducible and Validated with Bulk RNA-seq

- CTA performs with high reproducibility on FFPE

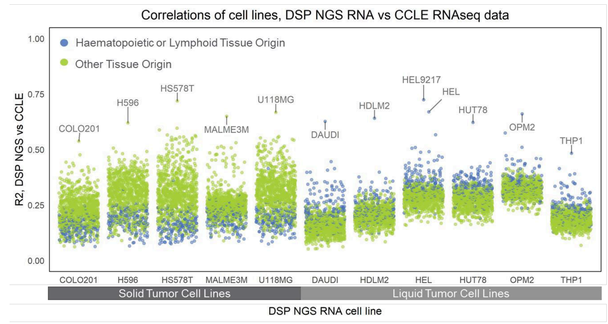


- CTA shows high concordance of GeoMx DSP counts with bulk RNA-seq*

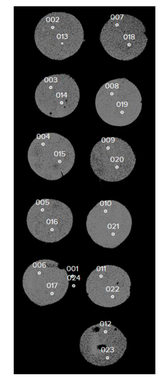


*data source from Cancer Cell Line Encyclopedia

CTA Shows High Specificity, Distinguishing the Correct Cell Line Expression Profile from > 1000 Cell Lines



200um AOIs in cell pellet array



- 1,388 genes, 1,019 cell lines compared
- Both HEL and HEL9217 are human erythroleukemia lines

Direct Comparison of GeoMx DSP to Bulk Gene Expression Measurements in Human FFPE Tissue Sections

Table 1: Datasets used in this study

FFPE source	Tissue type	Patients/ samples	AOIs per tissue	AOI types	Genes (DSP)	enrichment method (RNAseq)	Genes (IO360)	Genes common to all methods
Bladder	bladder cancer	22	12-26	segmented, geometric	156	polyA capture	784	107
Prostate	prostate cancer metastases (liver, lymph node)	4	24-48	segmented, geometric	1,412	polyA capture	N/A	1,408
Internal	tonsil	3	48-96 (gridded)	50µm and 300µm squares	96	rRNA depletion	N/A	96

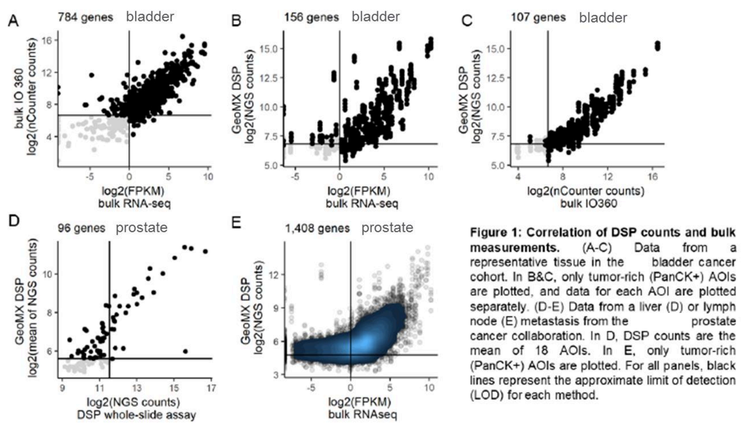


Figure 1: Correlation of DSP counts and bulk measurements. (A-C) Data from a representative tissue in the bladder cancer cohort. In B&C, only tumor-rich (PanCK+) AOIs are plotted, and data for each AOI are plotted separately. (D-E) Data from a liver (D) or lymph node (E) metastasis from the prostate cancer collaboration. In D, DSP counts are the mean of 18 AOIs. In **E**, only tumor-rich (PanCK+) AOIs are plotted. For all panels, black lines represent the approximate limit of detection (LOD) for each method.

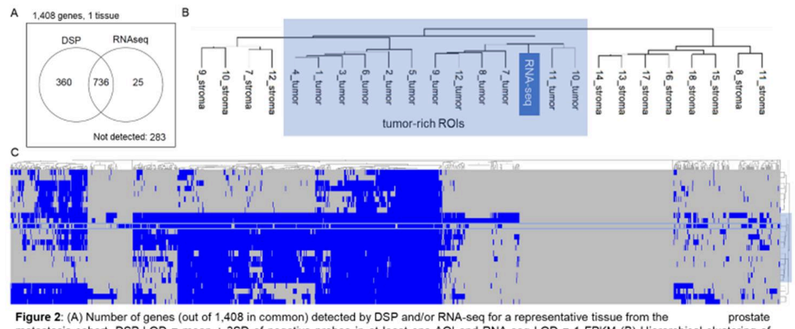


Figure 2: (A) Number of genes (out of 1,408 in common) detected by DSP and/or RNA-seq for a representative tissue from the prostate metastasis cohort. DSP LOD = mean + 3SD of negative probes in at least one AOI and RNA-seq LOD = 1 FPKM (B) Hierarchical clustering of ROIs (enlarged from (C)); rightmost sample corresponds to top row of heatmap. (C) Detection heatmap for the tissue shown in A. Blue indicates >LOD and grey indicates < LOD. Each row represents one DSP AOI or bulk RNA-seq data (boxed); each column represents one gene.

Conclusion

1. GeoMx® DSP with NGS readout offers flexibility and automation for a wide range of customer applications and workflows.
2. The Cancer Transcriptome Atlas enables *in situ* RNA expression profiling with 8000+ probes that represent 1800+ genes involved in immuno-oncology pathways. The targeted panel is designed and validated for high performance on FFPE tissue sections.
3. The Cancer Transcriptome Atlas offers high sensitivity for genome-scale expression profiling while preserving critical information about tissue architecture.

www.nanostring.com | info@nanostring.com | @nanostringtech

Margaret Hoang, Michelle Kriner, Zoey Zhou, Kristina Sorg, Erin Piazza, Joseph Beechem*
NanoString Technologies, Inc.
*Correspondence: jbeechem@nanostring.com

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