#440 - The Development and Performance of the GeoMx® Spatial Proteogenomic Workflow for the Detection of Both RNA and Protein on a Single FFPE Slide

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Introduction

The GeoMx® DSP enables spatially resolved, high-plex digital quantitation of proteins (≥ 147-plex) and RNA (up to 18,000- plex) from FFPE or FF samples[1-3]. This technology utilizes unique affinity reagents antibodies for protein or ISH probes for RNA coupled to UV photocleavable oligonucleotide barcodes. Tissue samples are co-incubated with these affinity reagents and fluorescent markers, then subsequently imaged using fluorescence microscopy. Oligonucleotide barcodes are then precisely liberated from any area of interest (AOI) with UV-light, collected and quantified with either an nCounter® Platform or Next-Generation Sequencer (NGS). To fully capture the biological processes that control transcription, translation and protein turnover, the individual RNA and protein datasets can be merged for multiomic analysis. However, technical variations stemming from section-to-section variability and precisely matching ROIs across multiple slides, needs to be taken into consideration when analyzing data. To gain deeper insight and control for these variables, multimodal omics has been used as an alternative approach, which pertains to the simultaneous, co-detection of multiple 'omes' in a single sample. Integration of a multimodal omic approach into the GeoMx workflow would enable a deeper characterization of limited and precious biological samples. Furthermore, the simultaneous assessment of RNA and protein from a single AOI would reduce technical variation associated with two separate, single analyte workflows. To expand upon GeoMx DSP capabilities, we have developed a novel co-detection workflow for NGS readout that allows for the profiling of both RNA and protein from the area of interest (AOI) on a FFPE tissue section[4]. Here we describe the technical development, performance, and application of the Spatial Proteogenomic workflow using high plex GeoMx Human/Mouse Protein Assays and the GeoMx Human/Mouse Whole Transcriptome Atlas (GeoMx Hu/Mm WTA).

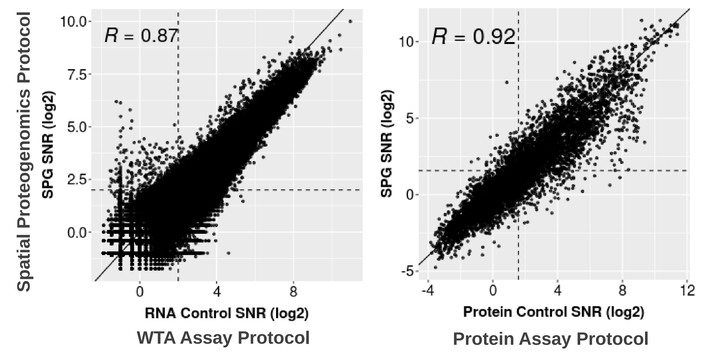
GeoMx Spatial Proteogenomics - High-plex Protein and RNA detection on a Single Slide

A

147-Plex Protein Panel for NGS

GeoMx Whole Transcriptome Atlas 18,000+ Gene Targets

With highly concordant performance to the single analyte GeoMx WTA or NGS Protein Assays, GeoMx Spatial Proteogenomic workflow eliminates variation from differing cell populations across serial slides



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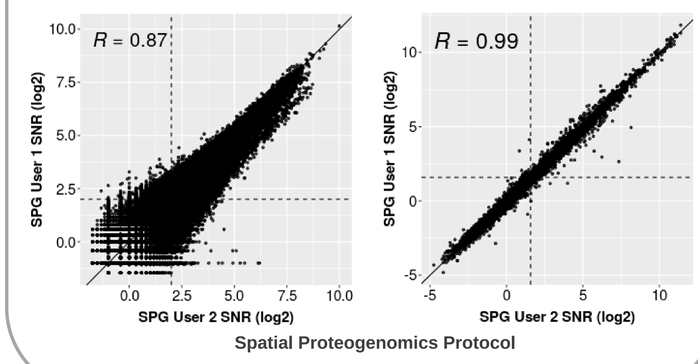
User friendly GeoMx Spatial Proteogenomics Protocol results in highly concordant results from slide to slide and user to user.



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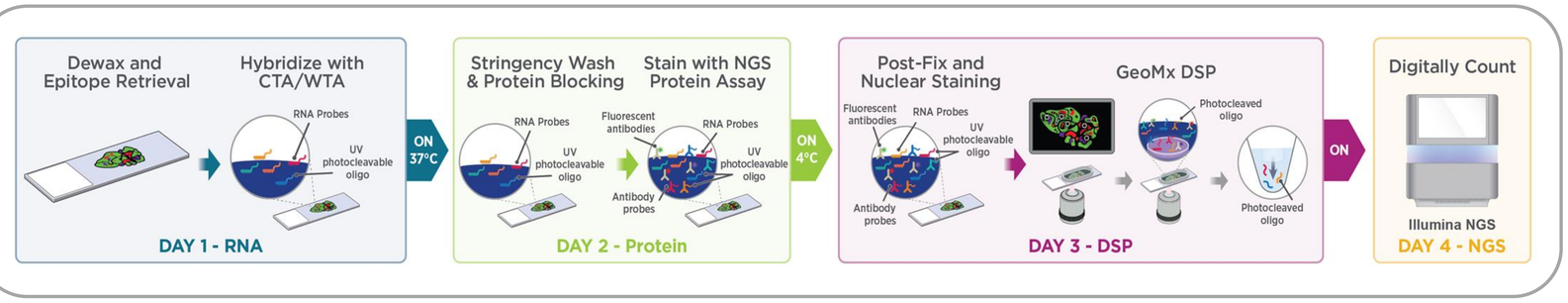
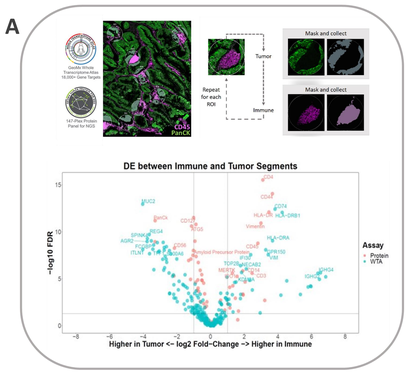


GeoMx Spatial Proteogenomic Workflow. The assay takes 4 days from slide prep to data analysis and is only compatible with NGS readout. Prior to staining, samples are subjected to a two-step epitope retrieval process involving HIER under basic conditions followed by proteolytic digestion with 0.1 ug/mL ProK.

Detect high plex RNA & Protein with spatial resolution in segmented Colorectal Cancer

A



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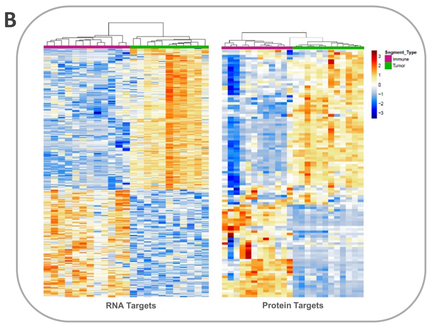

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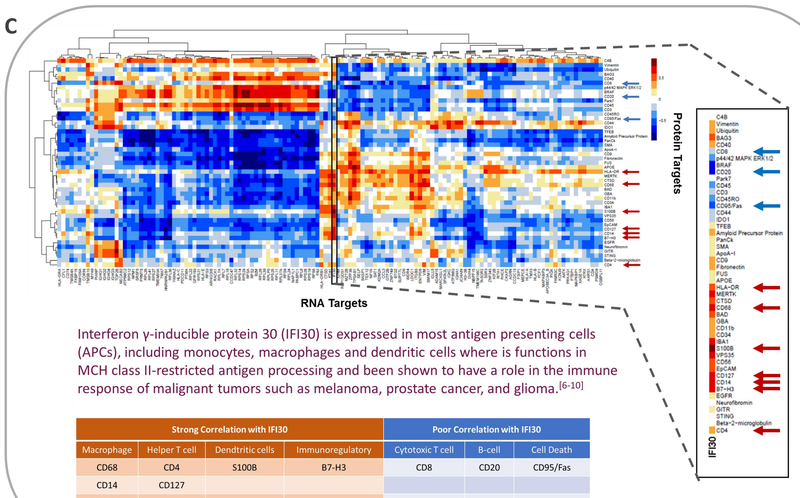


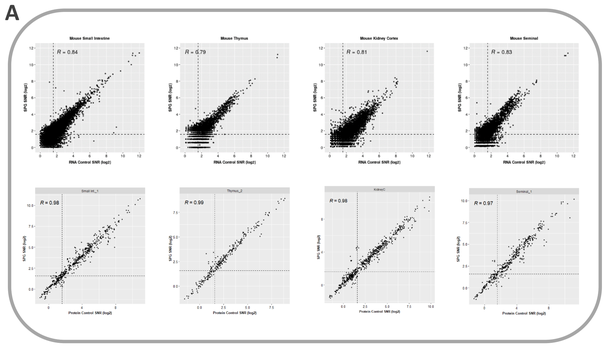
Interferon γ-inducible protein 30 (IFI30) is expressed in most antigen presenting cells (APCs), including monocytes, macrophages and dendritic cells where is functions in MCH class II-restricted antigen processing and been shown to have a role in the immune response of malignant tumors such as melanoma, prostate cancer, and glioma.[6-10]

Strong Correlation with IFI30				Poor Correlation with IFI30		
Macrophage	Helper T cell	Dendritic cells	Immunoregulatory	Cytotoxic T cell	B-cell	Cell Death
CD68	CD4	S100B	B7-H3	CD8	CD20	CD95/Fas
CD14	CD127					
HLA-DR						

Spatially resolved high plex RNA and Protein expression co-detection in distinct CRC tumor or immune segmented areas of interest. (**A**) Colorectal cancer FFPE sections were stained with GeoMx Hu WTA, 15 stacked GeoMx Human Protein Modules (147-plex) and fluorescently stained for PanCK⁺ (tumor, green) and CD45⁺ (immune, magenta). ROIs were segmented into marker specific AOI. Combined volcano plot of differentially expressed Protein and RNA expression. (**B**) Unsupervised hierarchical clustering of RNA or Protein targets in immune and tumor segments. (**C**) Correlation between RNA and Protein targets above background in immune segments. Unsupervised hierarchical clustering analysis revealed distinct patterns of correlation (red arrows) and anticorrelation (blue arrows).

GeoMx Spatial Proteogenomics as a one slide high plex multi-analyte solution for mouse

A



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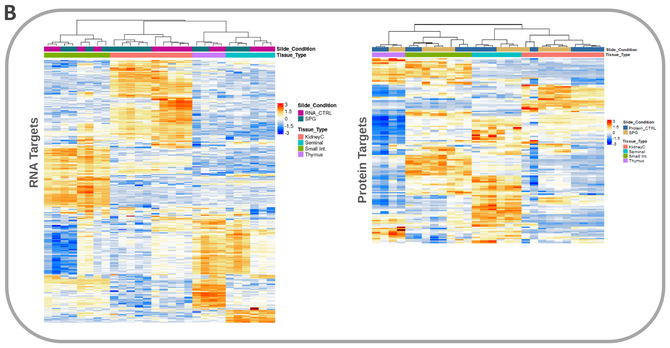
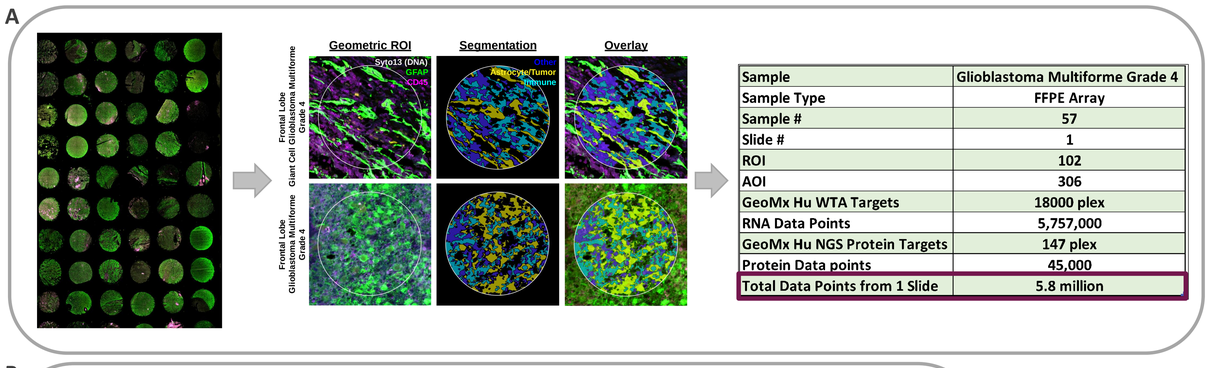


Spatial Proteogenomics across Mouse Tissue types. High plex spatial proteogenomic characterization of Mouse tissues with circular matched ROIs. (**A**) FFPE sections were stained with GeoMx Mm WTA (RNA control), 15 stacked GeoMx Mouse Protein Modules (137-plex) (protein control), or both analytes simultaneously with the spatial proteogenomic workflow. (**B**) Unsupervised hierarchical clustering of top 500 RNA targets and unsupervised hierarchical clustering of protein targets.

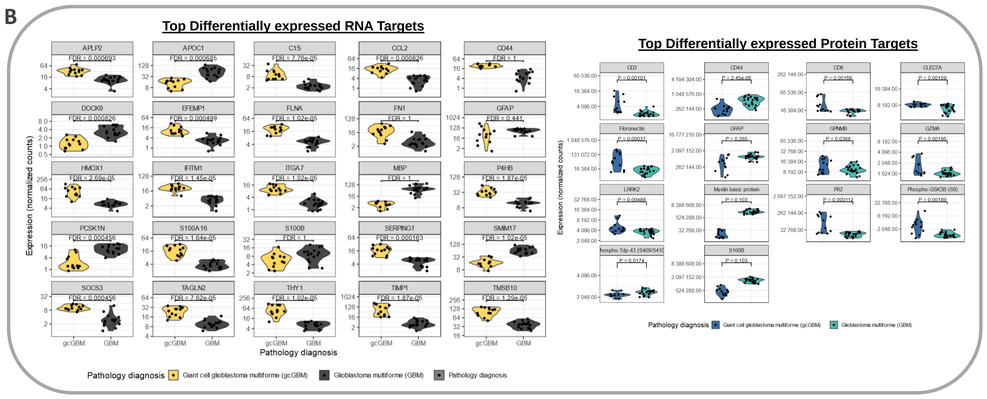
Increased T-cell infiltration observed in Frontal Lobe Giant Cell Glioblastoma vs Glioblastoma

A



Sample	Glioblastoma Multiforme Grade 4
Sample Type	FFPE Array
Sample #	57
Slide #	1
ROI	102
AOI	306
GeoMx Hu WTA Targets	18000 plex
RNA Data Points	5,757,000
GeoMx Hu NGS Protein Targets	147 plex
Protein Data points	45,000
Total Data Points from 1 Slide	5.8 million

B



Spatial Proteogenomic exploration of Glioblastoma multiforme Grade 4.
(A) 57 cores across 26 distinct sample sources and multiple GBM types. ROI were segmented into CD45⁺, GFAP⁺, or CD45⁻/GFAP⁻. Statistics from a single slide and a single GeoMx Spatial Proteogenomics run.
(B) Top differentially expressed RNA and Protein Targets from Frontal Lobe Giant Cell Glioblastoma Multiforme as compared to Glioblastoma Multiforme, all Grade 4. Analysis carried out by the Nanostring Spatial Data Analysis Service (sDAS).

Conclusion

- The GeoMx Spatial Proteogenomic Workflow allows for the co-detection of proteins (≥ 147-plex) and RNA (up to 18,000-plex) from a single FFPE sample
- Able to resolve distinct tumor or immune targets when the Spatial Proteogenomic Workflow is used in conjunction with the segmentation capabilities of the GeoMx
- The advantages Spatial Proteogenomic Workflow
  - Enables a deeper characterization of limited and precious biological samples
  - Able to profile both analytes from identical cell population
  - Eliminates technical variability associated with section-to-section variation and matching ROIs across multiple slides

References

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