Abstract Control Number: 3277:The Development and Performance of the Spatial Proteogenomic GeoMx® Workflow for the Detection of Both RNA and Protein on a Single FFPE Slide

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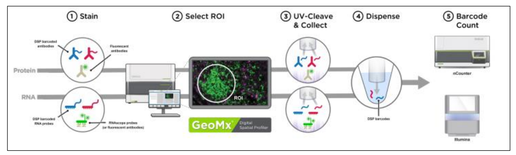
Introduction

The GeoMx ® DSP enables spatially resolved, high-plex digital quantitation of proteins (≥ 100-plex) and RNA (up to 21,000- plex) from FFPE or FF samples (**A**) [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 (**B**). However, technical variations stemming form 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 and performance of the Spatial Proteogenomic Workflow on cell pellet array (CPA) and various tissues using a high plex GeoMx Protein Assay and either the GeoMx Cancer Transcriptome Atlas (GeoMx CTA) or GeoMx Human Whole Transcriptome Atlas (GeoMx Hu WTA).

A



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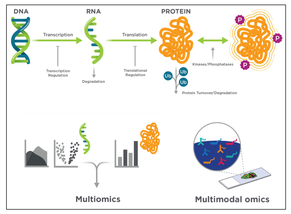
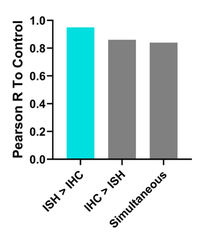


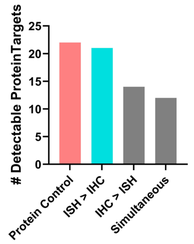
Impact of Staining Order on Protein Detection

6 Stacked GeoMx Human Protein Modules for NGS (59-plex)

- GeoMx Human NGS Core
- GeoMx Human NGS Immune Cell Typing Panel
- GeoMx Human NGS IO Drug Target Panel
- GeoMx Human NGS Immune Activation Status Panel
- GeoMx Human NGS Pan-Tumor Panel
- GeoMx Human NGS Myeloid Panel

Harsh ISH conditions (i.e. formamide) can have a negative impact on antigen-antibody interactions. We, therefore, screened three different "staining" strategies on FFPE cell pellet stained with a 6 stacked Human GeoMx Protein Module (59-plex). Optimal correlation and sensitivity to the control was observed with the sequential ISH > IHC staining approach and was therefore incorporated into the Proteogenomic Workflow.





Impact of varying ProK concentrations

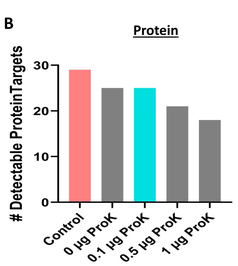
The impact of varying concentration of ProK on analyte detection was carried out on 45-cell pellet array (FFPE) pretreated under basic HIER conditions follow by proteolytic treatment. Samples were stained with GeoMx Hu WTA followed by a 6 stacked GeoMx Human Protein Module for NGS (59-plex) under Proteogenomic Workflow. (**A**) Pearson correlation on the log2 transformed SNR data between the proteogenomic workflow and the single analyte controls along with the CCLE RNAseq database. Plots represents the number of (**B**) detectable protein targets and (**C**) true positive detectable RNA targets.

A

ProK (µg/mL)	Protein	RNA	
		WTA Control	RNAseq CCLE[1, 2]
0	0.85	0.86	0.76
0.1	0.86	0.89	0.8
0.5	0.85	0.91	0.82
1	0.83	0.91	0.83

[1]Cancer Cell Line Encyclopedia (CCLE) [5]
[2]Pearson coefficient correlation between the CTA control and the CCLE data base is 0.86

B



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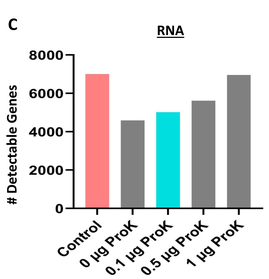
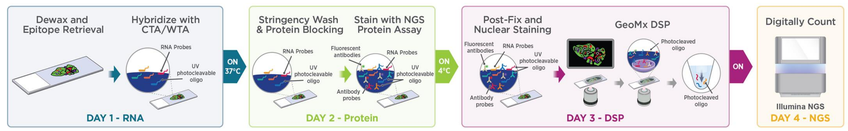


GeoMx Spatial Proteogenomic Workflow

GeoMx Spatial Proteogenomic Workflow enables multiomodal omic profiling on a single slide. The assay takes 4 days, from slide prep to data analysis, to complete and is only compatible with NGS readout. Prior to staining, samples are subjected to a two-step ER process involving HIER under basic conditions followed by proteolytic digestion with 0.1 ug/mL Prok.



Performance on CPA

Assessment of spatial proteogenomic performance versus the respective RNA and Protein control on cell lines.
A 11-cell pellet array (CPA) was stained with GeoMx CTA under and 6 stacked GeoMx Human Protein Module for NGS (59-plex) under Proteogenomic and standard workflow conditions. (**A**) Cell line to cell line comparison of Protein Control to proteogenomic protein data. (**B**) Target to target comparison of Protein Control to proteogenomic protein data. (**C**) Cell line to cell line comparison of GeoMx Hu WTA data from RNA Control and proteogenomic workflow to entire CCLE RNAseq dataset [5]. Comparison of protein targets to respective GeoMx Hu WTA RNA target using the (**D**) Proteogenomic Workflow and (**E**) multiomic.

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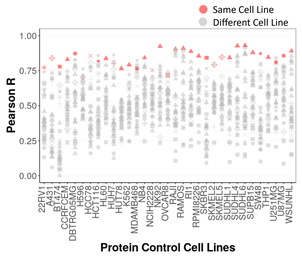

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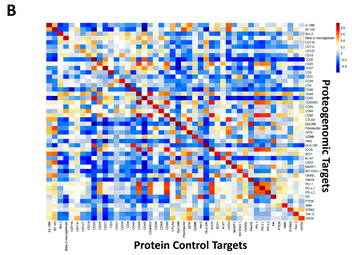

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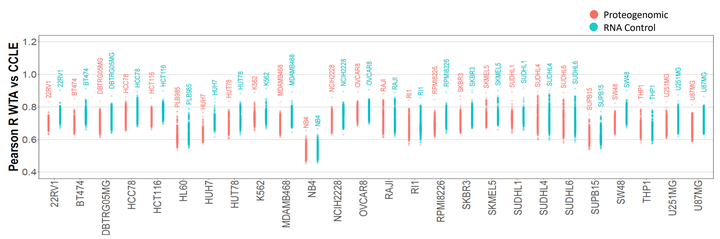

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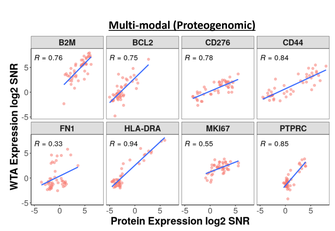

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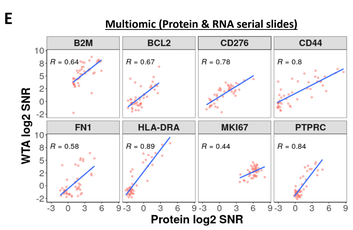

Performance on Non-small cell lung cancer (NSCLC)

Performance of proteogenomic workflow on non-small cell lung cancer (NSCLC). (**A**) FFPE sections of NSCLC were stained with GeoMx Hu WTA , 6 stacked GeoMx Human Protein Module (59-plex) for NGS and antibodies against PanCK (cyan, tumor) and CD45 (magenta, immune). ROIs of 100 µm in diameter were matched across all slide conditions and tissue sections. The average number of targets above the detection threshold for (**B**) protein and (**C**) genes targets for each region profiled by the single analyte Control or Proteogenomic Workflow. (**D**) Comparison of RNA targets observed in both RNA and Proteogenomic Workflow. (**E**) ROI to ROI comparison of GeoMx Hu WTA data. Pearson R between log2 SNR transformed data for each ROI from the RNA Control slide were calculated against all the ROIs in the Proteogenomic slide. Unsupervised hierarchical clustering was performed on the R values

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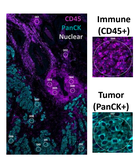

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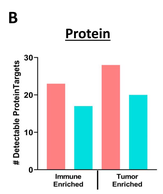

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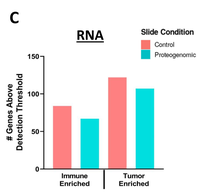

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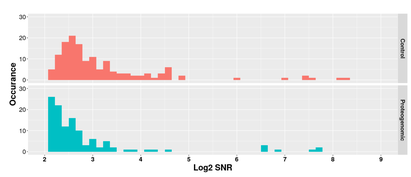

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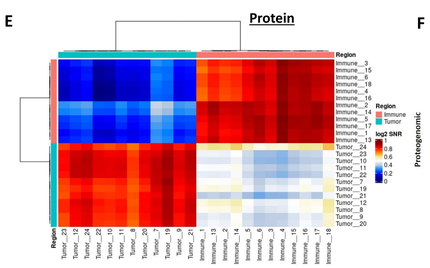

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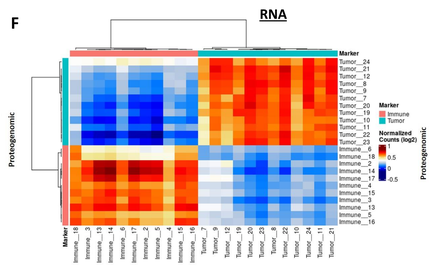

Spatial Performance on Colorectal Cancer

Spatial resolution of distinct tumor or immune RNA protein targets when using the proteogenomic workflow. Multiplexed protein and RNA characterization of CRC sample with 300 µm circular ROIs segmented into immune and tumor regions. (**A**) Colorectal cancer FFPE sections were stained with GeoMx Hu WTA, (**B**) The 15 stacked GeoMx Human Protein Modules (147-plex) and antibodies against PanCK (tumor, green) and CD45 (immune, magenta). Twelve circular ROIs of 300 µm in diameter were segmented into marker specific AOI. (C) Combined volcano plot of Protein and RNA expression. All immune segments were compared to all tumor segments for Protein and RNA targets above background. (**D**) Concordance between matching protein and RNA targets. Correlation between Proteogenomic RNA and Protein targets above background in (**E**) immune and (**F**) tumor. Hierarchical clustering analysis was revealed distinct patterns of correlation (red arrows) and anticorrelation (blue arrows).

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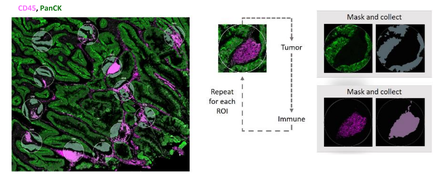

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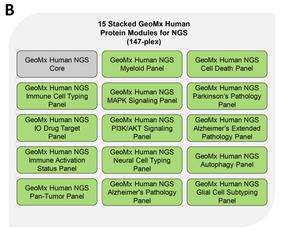

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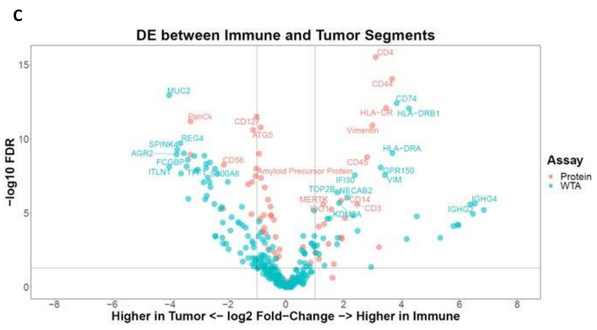

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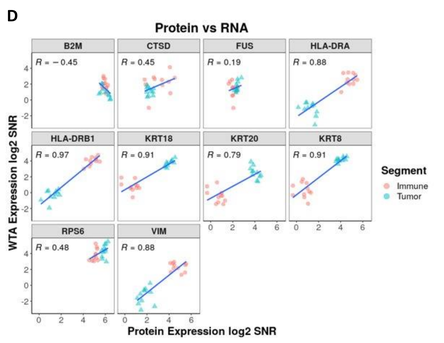

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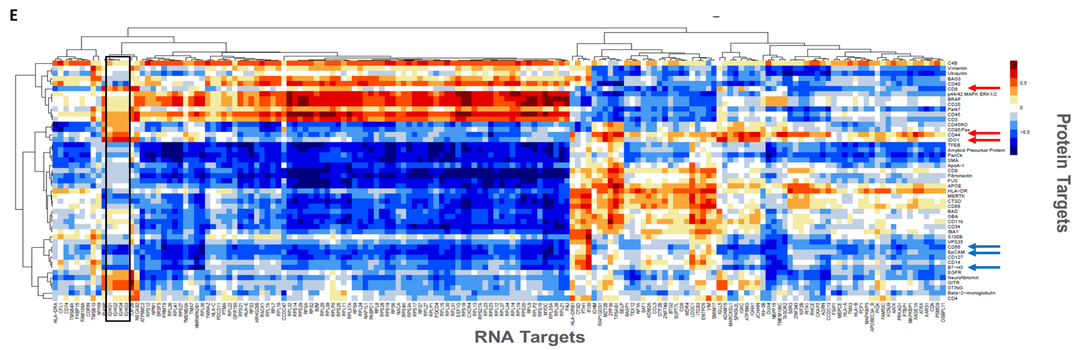

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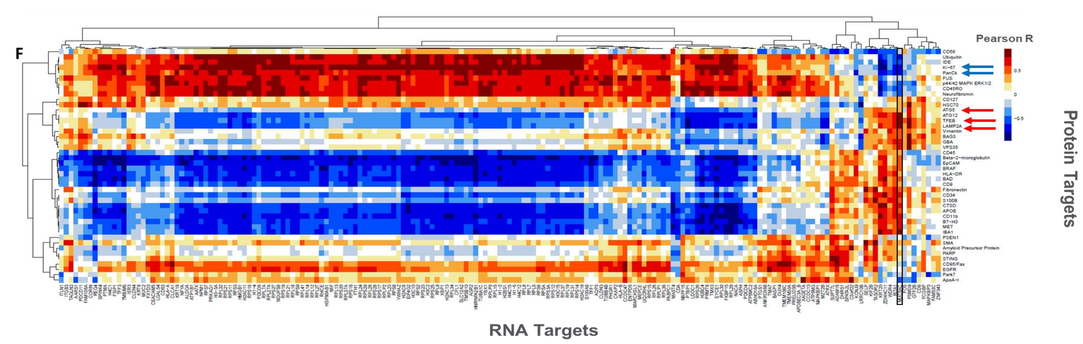

Conclusion

- The GeoMx Spatial Proteogenomic Workflow allows for the co-detection of proteins (≥ 100-plex) and RNA (up to 21,000-plex) from a single FFPE sample
- The performance of the Proteogenomic Workflow has been confirmed on various cell pellets and tissues
- Able to resolve distinct tumor or immune targets when the Proteogenomic Workflow is used in conjunction with the segmentation capabilities of the GeoMx
- The advantages 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

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