#514 nanoString High throughput spatial-omics sample processing of archival FFPE specimens at the whole transcriptome level using digital spatial profiling

Margaret Hoang¹, Katrina van Raay¹, Gokhan Demirkan, Joseph M Beecham^{1*}, ¹. NanoString[®] Technologies Inc, Research & Development, Seattle, WA, USA, *Correspondence: jbeechem@nanostring.com

NanoString Technologie 530 Fairview Avenue North, Seattle. WA 98109

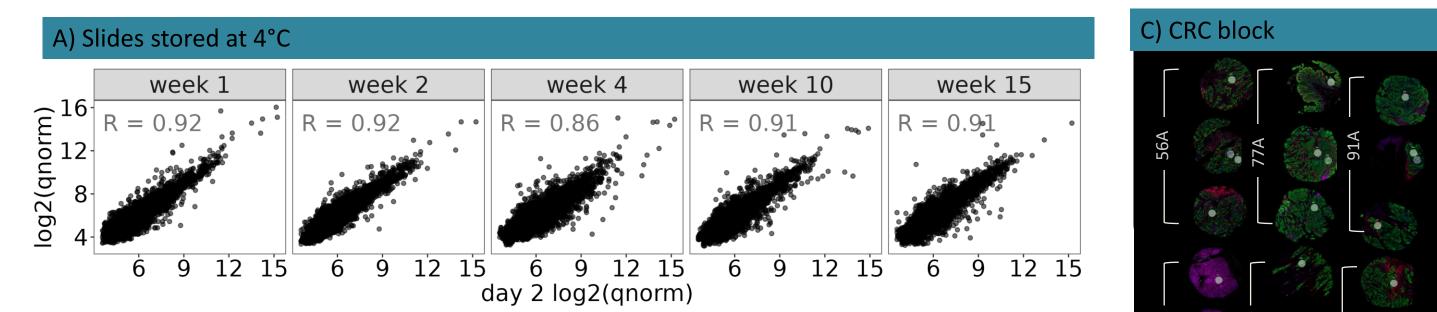
Abstract

Spatially resolved whole transcriptomes offer promising new insights into the study of human disease from archival FFPE specimens, including mechanism of drug action, biomarker discovery, and immune response. However, an emerging unmet need in spatial-omics is the ability to standardize and process patient samples from large cross-sectional or longitudinal cohort studies. Here we test batch slide processing and storage of whole transcriptome stained slides to maximize the run rate through one GeoMx[®] Digital Spatial Profiler (DSP) instrument. We processed 28 slides using the standard automated slide preparation protocol for in situ hybridization of 18,000+ probes to the whole transcriptome. For biosamples, we use standard reference control samples for the testing, comprised of 14 FFPE serial sections of a tissue microarray (TMA) with 10 different human tissue types and 14 FFPE serial sections of a cell pellet array (CPA) with 11 different human cell lines. After batch slide preparation, we ran the GeoMx DSP instrument with two slide replicates, each of the TMA and CPA, as Day 1 of the standard workflow with no storage and then stored the whole transcriptome hybridized tissues for subsequent runs on Day 2, 3, 4, 7, 14, and 21 as the test workflow with storage. Results show high concordance of gene expression counts of the 18,000+ probes between spatially matched regions-of-interest between all days with and without storage (Pearson R > 0.9). This concordance was similar to the observed slide-to-slide variation between replicates within each day. We also find throughout the storage time course a similar number of genes detected in tissues and similar fluorescence intensity of morphology marker antibody staining (PanCK, CD68, CD45). Furthermore, we show that gene expression profiles drive unsupervised hierarchical clustering of tissues and cell lines rather than days spent in storage. We observed a modest reduction of fluorescent intensity of the nuclear stain Syto13 with slide storage up to 21 days, but the nuclear stain remained functional with similar numbers outputted from the nuclei counting algorithm over time. Our results show a high throughput, scalable workflow that enables spatially resolved whole transcriptomes of large patient cohorts (100+) with archival FFPE tissue within less than a month.

Sectioned slides can be stored at RT or at 4°C for at least 15 weeks



An FFPE block made up of colo-rectal cancer samples from ten patients was sectioned on Day 0 and allowed to dry at room temperature for 2 days. On Day 2, two slides were run on GeoMx DSP using the human Whole Transcriptome Atlas panel and the remaining serial sections were stored dry at either 4°C or at room temperature with desiccator packets, after which slides were prepared using the Leica semi-automated protocol and hybridized with WTA.



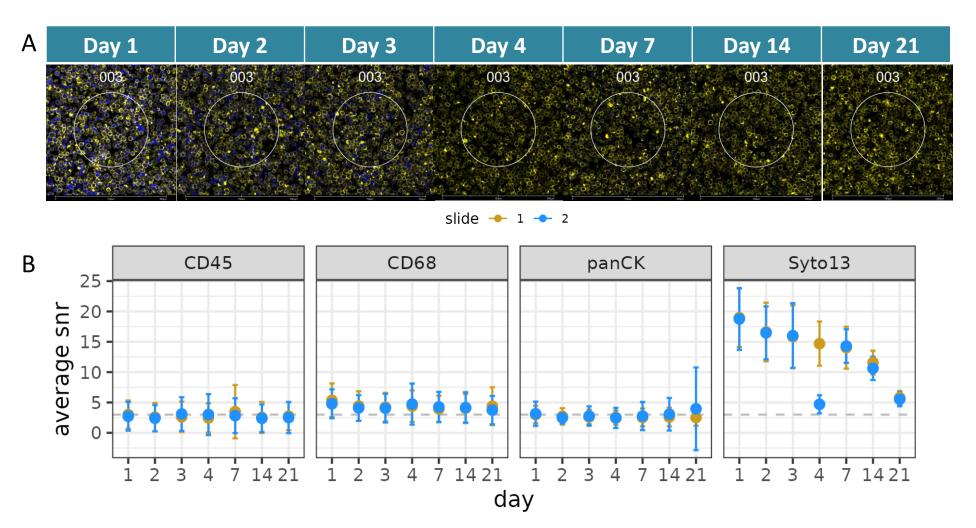
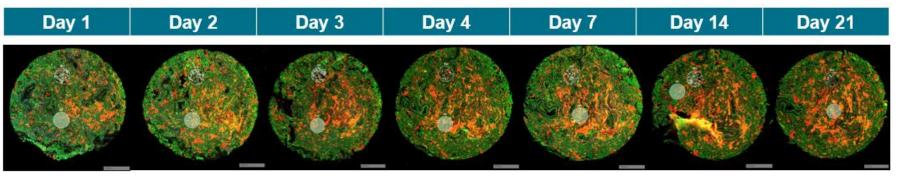
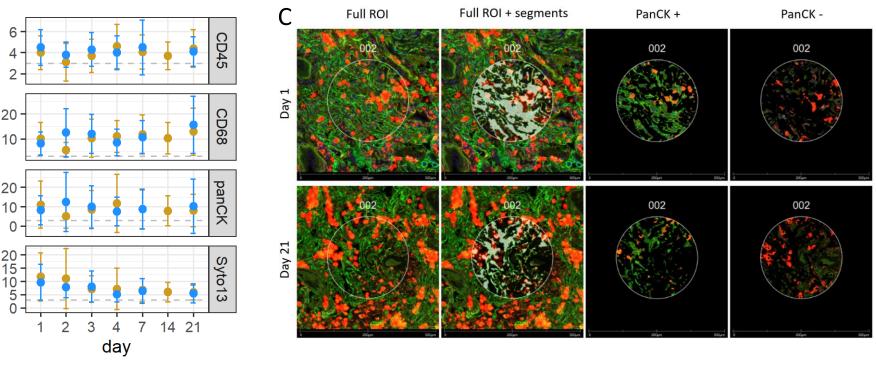


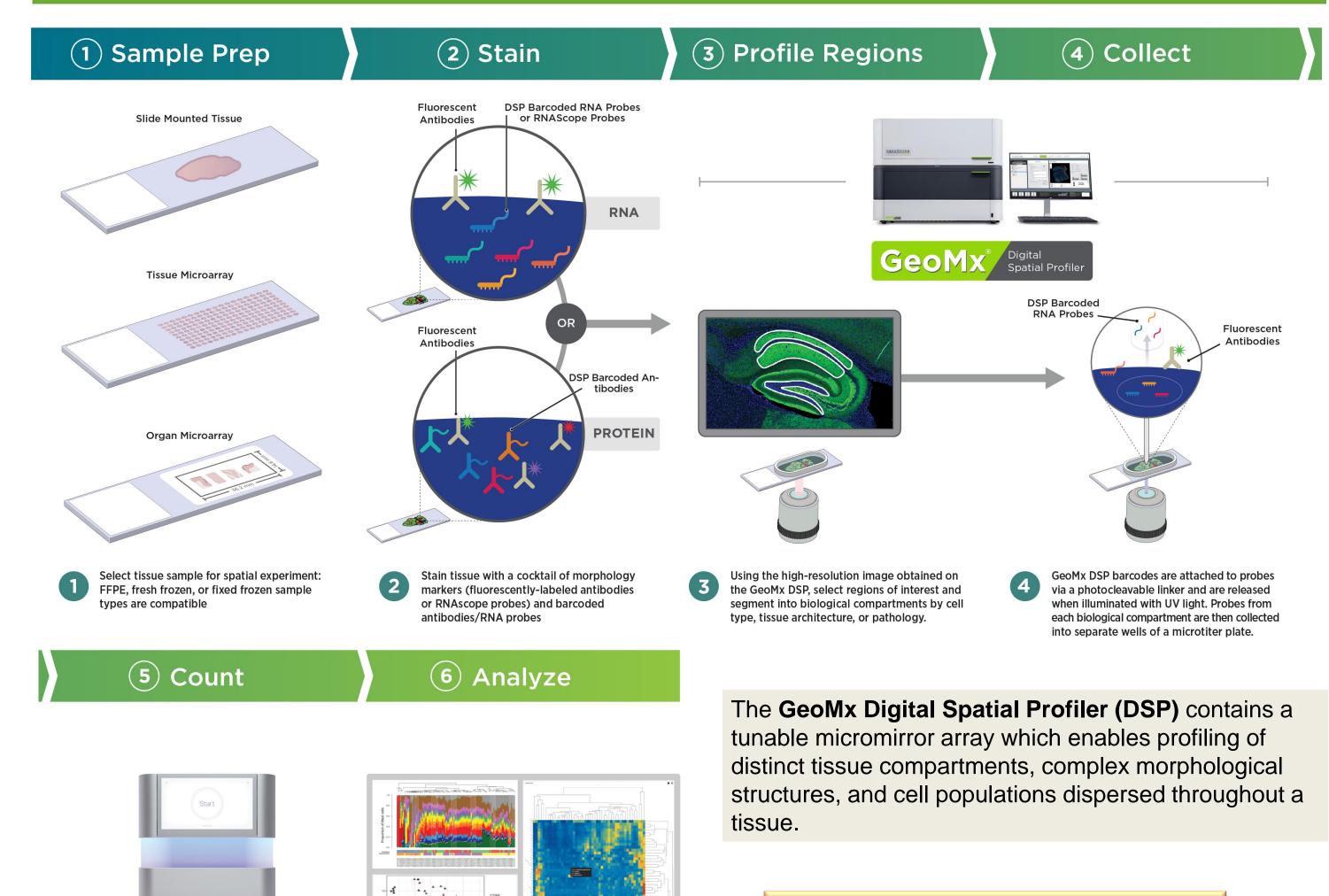
Figure 4: . A) Images of Daudi cell line over 21 days. Morphology markers are as follows: Syto13 (blue), PanCK (green), CD45 (yellow), and CD68 (red). B) Average signalto-noise ratio (SNR) for each CPA slide each day for morphology markers. The dashed line (y=3) denotes the minimum threshold for SNR for segmentation. Error bars are +/standard deviation.

Figure 5: A) Images of lung tissue over 21 days. Morphology markers are as follows: Syto13 (blue), PanCK (green), CD45 (yellow), and CD68 (red). B) Average signal-to-noise ratio (SNR) for each TMA slide each day for morphology markers. The dashed line (y=3) denotes the minimum threshold for SNR for segmentation using that marker. C) Segmentation on PanCK in one ROI in Lung tissue at Day 1 and Day 21. Panel shows full ROI (PanCK in green and CD68 in red), ROI with segments denoted, PanCK+ segment, and PanCK-





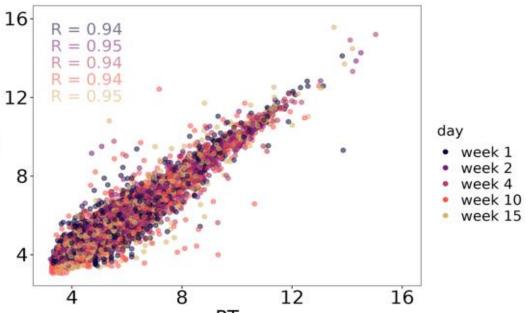
GeoMx[®] DSP enables direct in situ expression profiling

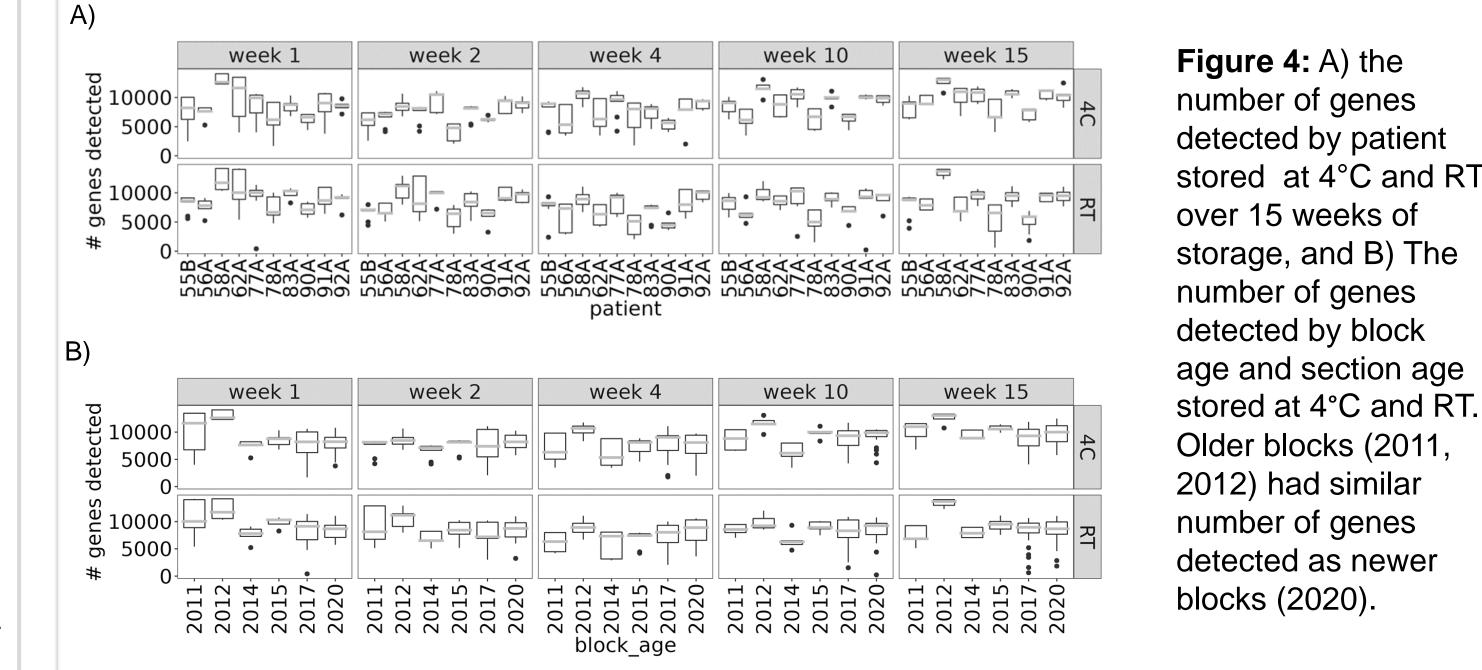


V06 Slides stored at room temperature (RT) week 1 week 2 week 10 week 15 week 4 = 0.93R = 0.93<u>5</u>12 12 15 6 9 12 15 6 9 12 15 6 9 12 15 12 15 D45 PanCK CD68 Syto13 day 2 log2(qnorm)

Figure 2: Correlations between the freshest section (Day 2) and all other section ages (weeks 1, 2, 4, 10, 15) stored at 4°C (A) or at room temperature (B). An annotated image of the CRC block with morphology makers is shown in (C).

Figure 3: Correlations of normalized gene expression data between slides stored at room temperature and 4C were high (Pearson R > 0.94) regardless of time spent stored.





Bulk slide preparation does not negatively affect gene expression data

Morphology markers still useable 21 days after staining

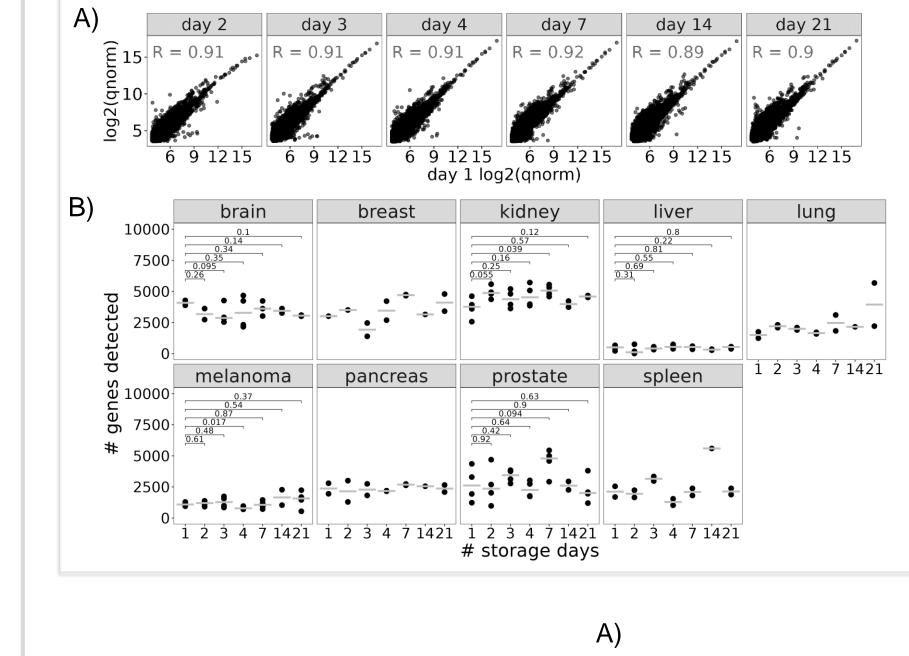
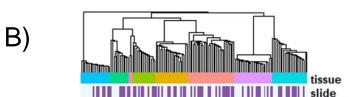
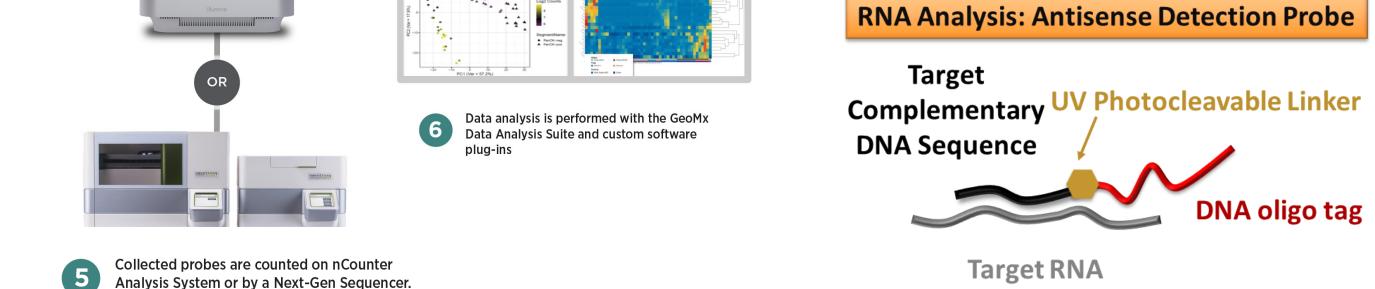


Figure 5: Reproducibility of Q3 counts between day 1 and each other timepoint in TMA slides. B) Data indicates that FFPE slides can be stored for 21 days poststaining without reducing gene detection in TMA. Grey bar indicates median. P-values from unpaired t-tests between day 1 and each other day are shown where there is data for at least 3 ROIs







Tagged Oligonucleotide Chemistry

GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue

Bulk sample preparation increases throughput and reduces hands-on time

Flexibility with long-term tissue section and stained slide storage are important aspects to consider when planning a large study that seeks to minimize sample processing batch effects. We investigated the impact of storing both sectioned tissues and RNA-stained slides for multiple weeks on the reproducibility and quality of DSP RNA count data, and identified two stopping points in the sample preparation process that did not negatively affect gene expression data.

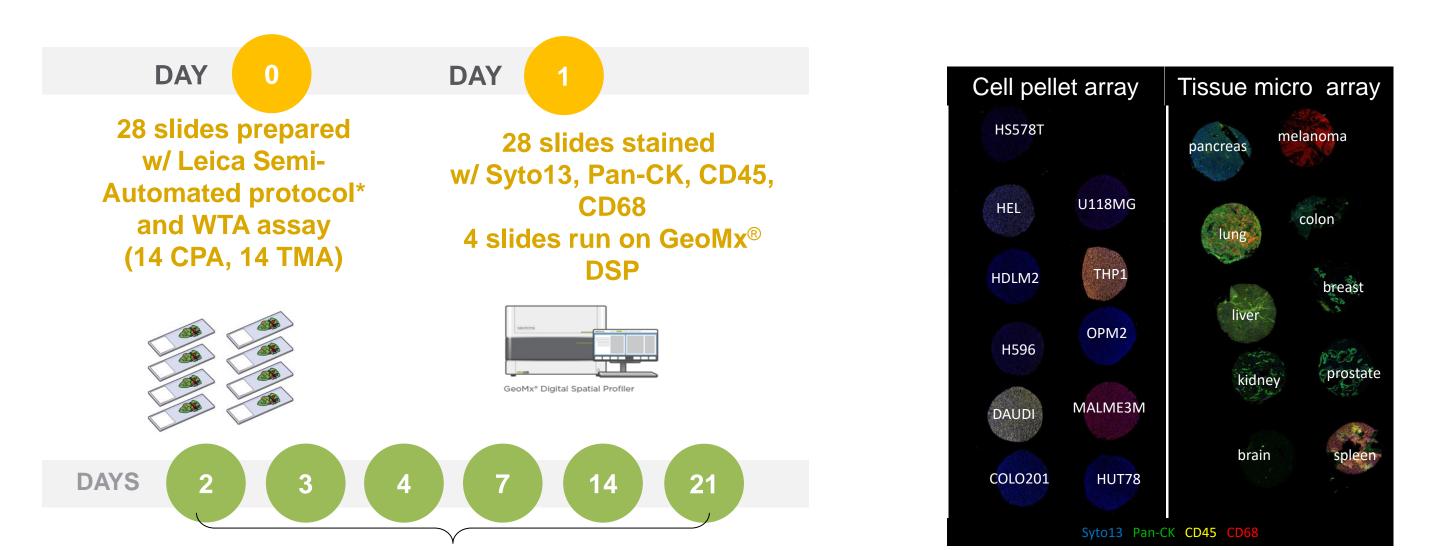


Figure 1 : We have identified two areas within the GeoMx Sample Prep workflow that are good stopping points after largescale sample processing samples posttissue sectioning (1) and post-slide staining

ANSCRIPTO

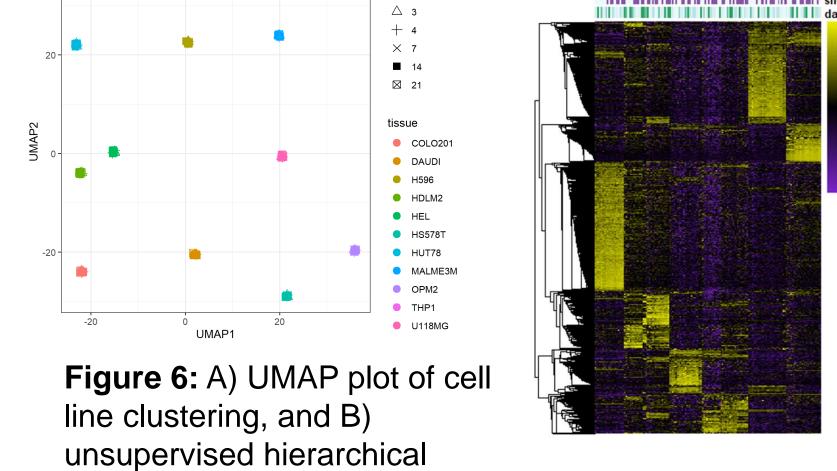
These data show that FFPE blocks up to 11 years old may be sectioned in bulk and stored for up to 15 weeks at either 4°C or RT without seeing a reduction in gene counts.

Bulk slide preparation increases efficiency without decreasing data quality



Cell line or tissue, not day, drive primary clustering. We used a Uniform Manifold **Approximation and Projection** (UMAP) for dimension reduction in the cell line dataset and found that cell line drives primary clustering (Figure 6A). Similarly, tissue drives primary clustering in an unsupervised hierarchical heatmap rather than days spent in storage (Figure 6B).

segment.

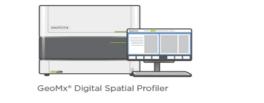


Conclusions

clustering of TMA

- Sectioned slides may be stored for up to 15 weeks without seeing a reduction in gene counts
- Block ages up to 11 years old did not influence number of genes detected
- Storage temperature did not affect gene expression data sectioned slides may be stored at either 4°C or RT
- Slides may be bulk prepared with probes and morphology markers and stored for up to 21 days in 2xSSC without seeing a decrease in gene

4 slides run on GeoMx[®] DSP each day



• Fourteen cell pellet array slides and 14 tissue micro array slides were prepared using the Leica semi-automated protocol and hybridized with huWTA.

- The following day, all 28 slides were stained with morphology markers.
- Four of these slides were then run on GeoMx DSP, while the remaining 24 slides were stored in 2x SSC in light-sealed containers at 4°C.
- Prepared slides were stored for 2, 3, 4, 7, 14, and 21 days and run on GeoMx DSP without any additional slide preparation.
- Bulk preparation of slides can reduce hands-on time and batch effects related to slide prep.

An 11-cell pellet array and 10-organ tissue micro array were used to cover a variety of FFPE tissue types. expression data

 Nuclei counting algorithms and segmentation algorithms remained functional 21 days after staining.

• Taken together with an efficient end-to-end workflow (see AGBT 2023 poster 127), increased throughput of 100+ slides per month is possible with bulk preparation of tissue sectioning and slide preparation.



FOR RESEARCH USE ONLY. Not for use in diagnostic procedures. www.nanostring.com | info@nanostring.com ©2023 NanoString Technologies, Inc.