Spatially resolved whole transcriptome analysis in mouse using the GeoMx[®] Digital Spatial Profiler

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Abstract

 The mouse Whole Transcriptome Atlas (MuWTA) targets >21,000 gene targets, 17 commonly used transgenes, and 210 negative controls. •Using cell and tissue arrays and sagittal brain sections we investigated

- Sensitivity and specificity
- •Fixed Frozen and FFPE correlations
- •The ability to characterize distinct gene expression profiles
- •The ability to utilize a variety of tissues •Together these results show that MuWTA is a powerful tool to profile any mRNA in multiple mouse tissue types and preparations



The presence or absence of a gene was defined by the limit of quantitation (LOQ) which was determined by calculating the geometric mean of the negative probes, multiplied by the standard deviation raised to the power of 2. Cell type deconvolution was performed using the SpatialDecon R package, using cell types characterized by scRNA-Seq from thalamus regions in Saunders et al., 2018, Cell.





Brain profiling with intelligent AOI selection enables distinct clustering of individual regions and identifies spatial variation in cell types

MuWTA uncovers structure-specific clustering and a spatial component to cell composition across a deeply profiled brain region

Regions within the thalamus, hippocampus, and fiber tracts, on two FxF sagittal brain sections were profiled by 200 micron square and custom polygon AOIs. AOIs annotated according to brain region (right). tSNE plot of AOIs reveals that distinct brain regions cluster together and correspond to unbiased cluster assignment by PCA (bottom) .

HPF-CA3so

tsne1

PF-PR

CA3sp

tsne2

HPF-DGmo



Dorsal AOIs

Plotting neuronal cell type abundance by spatial coordinates reveals cell type composition variation along the dorsal ventral axis

Ventral AOIs







Correlation matrix comparing the association of different cell types (following cell type deconvolution) in the thalamus. Groups of cell types that exhibit strong inverse spatial correlation are boxed in black dotted lines. Cell types that exhibit strong positive spatial correlations are boxed in gray.

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MuWTA recapitulates known biology in FFPE tissue

MuWTA captures biology across eight-organ array: 19,000+ genes detected across diverse tissues



Total number of genes detected by percent AOIs in which that gene was detected (left). Number of genes detected per AOI in different organ types (right).

ssGSEA Volcano Plot for Brain vs Not Brain

MuWTA gene expression and pathway analysis recapitulate known biology

- DSP counts from individual ROIs correlate better with RNAseg data from the same tissue type than dissimilar
- analysis shows the expected
- pathways in brain (right).

Higher in Brain <- NES EC -> Lower in Brain

Conclusions

- MuWTA shows high sensitivity and specificity, detects ~5,000-10,000 genes per AOI across a range of tissues
- MuWTA exhibits high concordance across AOI sizes, between sample prep types, and to RNA-Seq
- MuWTA recapitulates known biology in an organ array
- MuWTA leverages targeted AOI design to capture biological differences in distinct brain structures
- Cell deconvolution allows spatial correlations of cell populations

MuWTA detects biological differences between distinct anatomical regions of the same brain structure









Heatmap of genes differentially expressed between different brain regions (bottom). Profiled regions are colored **(top)** to match the heatmap.



