#492 Spatial whole transcriptome profiling of mouse organogenesis

Stephanie Zimmerman, Alexa Lasley, Michael Rhodes, Kit Fuhrman, Margaret Hoang, Nicholas Confuorto, and Joseph Beechem

NanoString Technologies, Seattle, WA

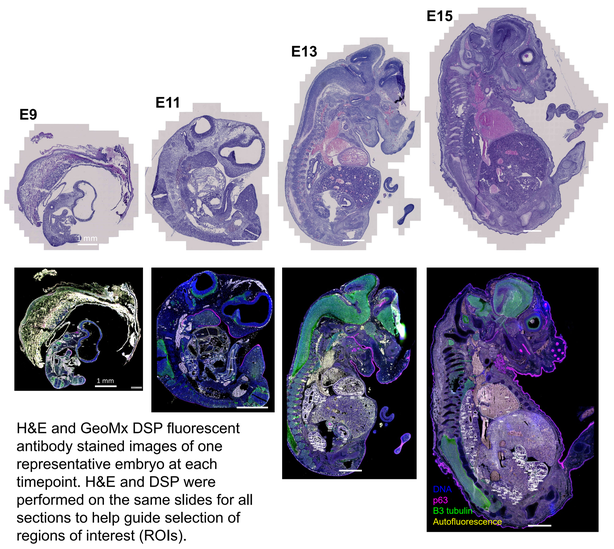
NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Abstract

Mammalian development is driven by key transcriptional regulators that are exquisitely specified in time and space. Insight into the transcriptional programs driving organogenesis have traditionally come from low-plex *in situ* methods and bulk or single cell transcriptomics of dissociated embryos. However, recent technological advances have made it possible to profile whole transcriptomes of defined regions of interest while retaining their spatial context. We used NanoString's GeoMx® Digital Spatial Profiler and the Mouse Whole Transcriptome Atlas to comprehensively profile anatomical substructures of nine organs from whole mounted fixed frozen mouse embryos (E9, E11, E13, and E15). Whole transcriptomes were collected from specific histological structures of 15 organs or their precursors. Similar cell types across organs (e.g. epithelium or mesenchyme) showed both shared and organ-specific gene expression profiles, and we identified both known and novel cell-type specific marker genes. Transcription factors were among the most differentially expressed genes in time and space. We characterized the expression pattern of key transcription factors driving gut development and identified novel temporally variable and substructure-specific expression. We also used our spatial data to localize and annotate unknown cell types identified in single cell RNAseq. These results provide a spatial and temporal atlas of the transcriptional programs governing key cell fate decisions during development.

The GeoMx Mouse Whole Transcriptome Atlas enables spatial gene expression profiling of organogenesis

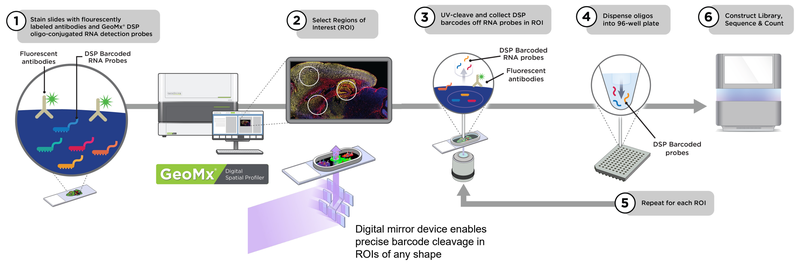
During development, major organ systems develop and expand in mid-gestation. We used GeoMx Digital Spatial Profiling (DSP) to perform spatial whole transcriptome profiling of 15 developing mouse organs at four embryonic timepoints, with regions of interest precisely drawn to capture specific histological structures.



H&E and GeoMx DSP fluorescent antibody stained images of one representative embryo at each timepoint. H&E and DSP were performed on the same slides for all sections to help guide selection of regions of interest (ROIs).

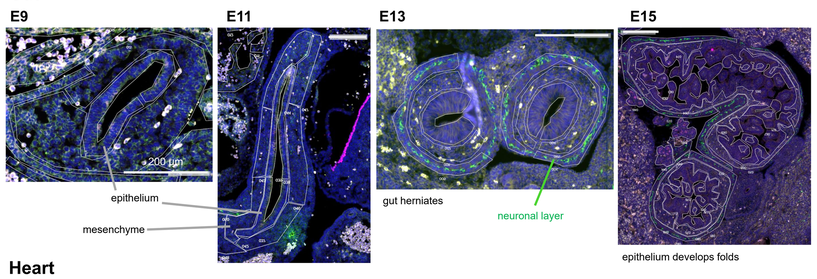
We profiled four fixed-frozen mouse embryos at embryonic days post-conception E9, E11, E13, and E15 (2-6 sections per timepoint). Because of their small size, E9 embryos were comprehensively profiled with ROIs covering the entire embryo. In total, we collected 733 ROIs spanning 15 major organs or their precursor structures.

GeoMx DSP with the Mouse Whole Transcriptome Atlas enables quantification of 20,175 protein-coding genes in precisely defined regions of interest (1)

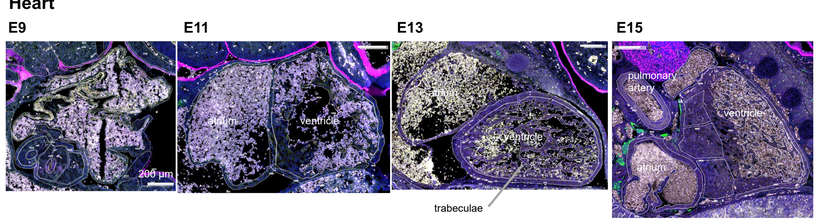


Regions of interest drawn to capture histological structures within organs

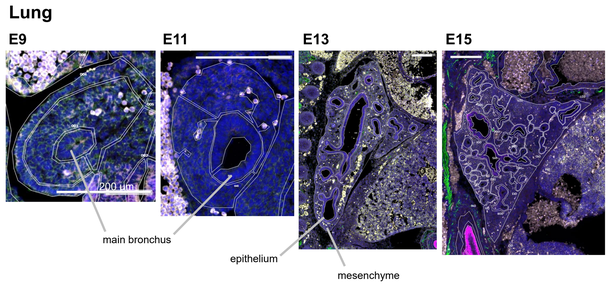
Gut



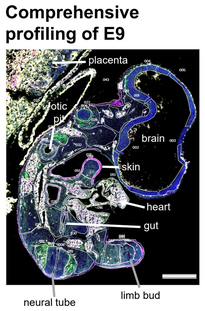
Heart



Lung



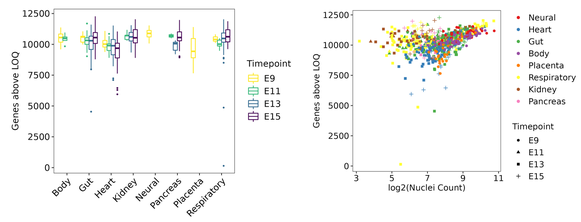
Comprehensive profiling of E9



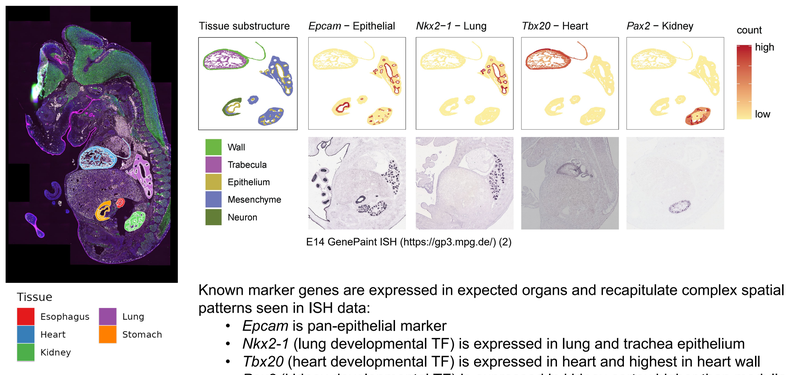
Robust and accurate gene detection across organs, structures, and timepoints

Robust gene detection across all organs and timepoints in a wide range of ROI sizes

We detected 18,045 genes above background in at least 1% of the 733 ROIs, with ~8000-9000 genes detected in each ROI. Gene detection rates were similar across all organs and timepoints profiled and in ROIs down to ~20 cells.



Validation of known tissue- and substructure-expressed genes comparing DSP of E13 embryos with public ISH data of E14 embryos



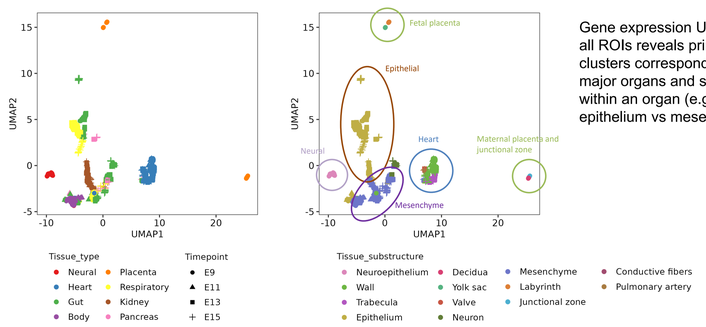
E14 GenePaint ISH (https://gp3.mpg.de/) (2)

Known marker genes are expressed in expected organs and recapitulate complex spatial patterns seen in ISH data:

- *Epcam* is pan-epithelial marker
- *Nkx2-1* (lung developmental TF) is expressed in lung and trachea epithelium
- *Tbx20* (heart developmental TF) is expressed in heart and highest in heart wall
- *Pax2* (kidney developmental TF) is expressed in kidney cortex higher than medulla

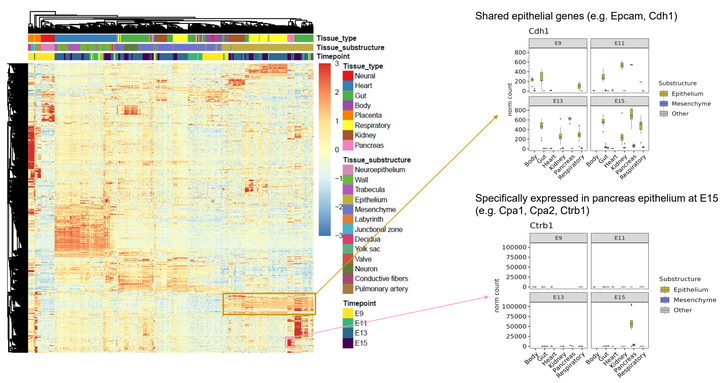
The global transcriptome of developing organs and organ structures

Tissue and tissue substructure drive primary clustering, with structures of the same origin clustering together



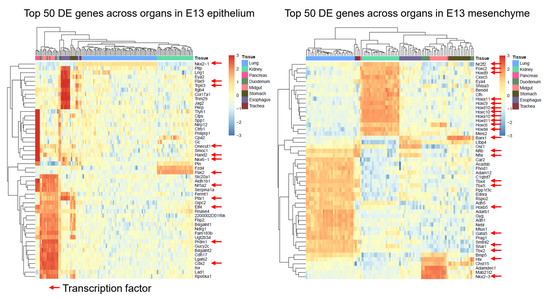
Gene expression UMAP of all ROIs reveals primary clusters corresponding to major organs and structures within an organ (e.g. epithelium vs mesenchyme)

Heatmap of the 2000 most highly variable genes across the dataset shows clustering primarily driven by spatial variation, but some genes are also temporally specific. For example, we identify genes highly expressed in epithelium of all tissues, as well as genes that are specifically expressed in the pancreas epithelium at E15.

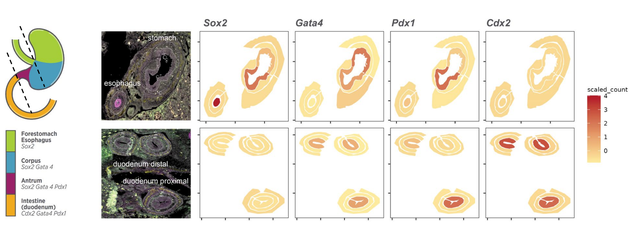


Spatially-specific genes include key developmental transcription factors

At each timepoint, we identified the most differentially expressed genes across organs in specific structures and found that transcription factors were among the most differentially expressed genes. Overall, we quantified 1121 of the 1151 protein-coding mouse transcription factors annotated in TFDB (97%) (3). At E13, we found that 642 TFs (56%) were significantly differentially expressed between organs, including known master regulators of organ development as well as many novel associations.

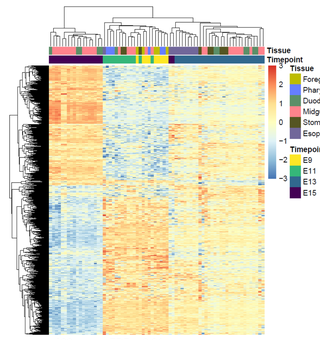


DSP data recapitulates the known expression of transcription factors that pattern gut epithelium development from anterior to posterior at E13 (4) but reveals novel spatial patterns – for example higher *Pdx1* expression in the liver-proximal duodenum.



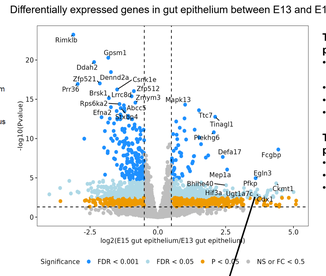
Identifying genes that change during development in specific organ structures

Gut epithelium, DE genes across timepoints



833 genes, FDR < 0.01

Differentially expressed genes in gut epithelium between E13 and E15



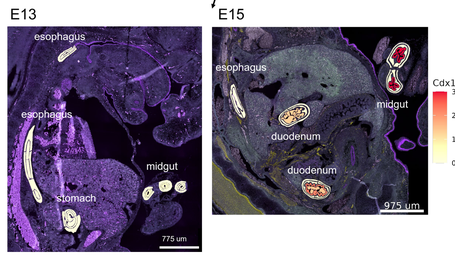
Top enriched Reactome pathways E15

- small molecule metabolic process
- lipid metabolic process
- immune response
- response to bacterium

Top enriched Reactome pathways E13

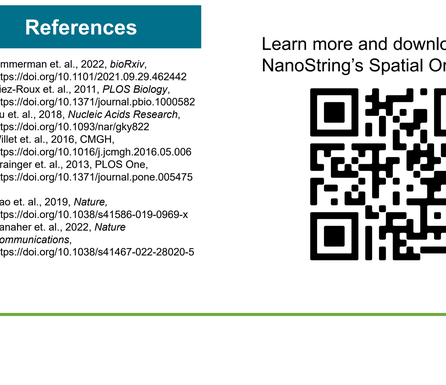
- cell projection organization
- cell junction organization
- regulation of canonical Wnt signaling pathway

We identified genes that change with time in specific organs or histological structures. For example, in the gut epithelium over 800 genes were differentially expressed across timepoints. Differentially expressed genes between E13 and E15 reveal an increase in metabolic and immune related genes. In addition, we find that the known adult intestine transcription factor *Cdx1* (5) specifically turns on in the posterior gut late in development, potentially mediating transcriptional changes that occur at E15.

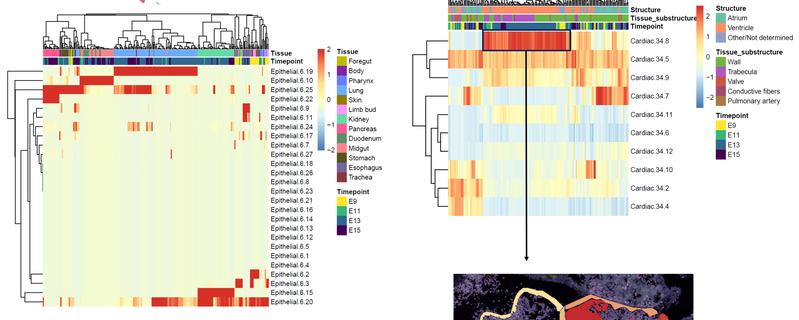


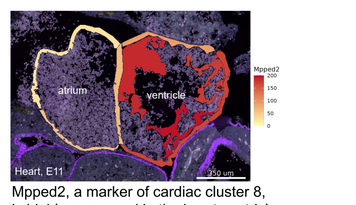
Localizing unannotated cell types from scRNAseq with spatial profiling

Mouse Organogenesis Cell Atlas main clusters and subclusters (6)



Large single cell RNAseq projects have collected gene expression data from millions of single cells in developing mouse embryos. However, the spatial context of these cells is unknown. In the Mouse Organogenesis Cell Atlas (6), 2 million cells from E9-E13 embryos are clustered based on gene expression. Main cell clusters are annotated based on the expression of marker genes, but sub-clusters remain unannotated. We used cell type deconvolution (7) to localize unannotatated epithelial and cardiac subclusters to regions profiled by DSP, revealing that many cell clusters are spatially-specific.





Deconvolution of epithelium ROIs (left) and heart ROIs (right) using gene expression profiles derived from epithelial and cardiac subclusters, respectively, reveals spatial location of specific cell types. For example, Epithelial cluster 22 is enriched in the pancreas epithelium, Epithelial cluster 15 in the kidney epithelium, Epithelial cluster 7 is enriched in the otic pit, Cardiac cluster 8 in the heart ventricle, and Cardiac cluster 7 in the heart atrium.

Mpped2, a marker of cardiac cluster 8, is highly expressed in the heart ventricle

Conclusions

- Developing mouse organs can be comprehensively profiled spatially with the GeoMx Mouse Whole Transcriptome Atlas
- GeoMx WTA reveals known and novel transcription factors and transcriptional programs that specify organ structures across space and time
- GeoMx WTA provides spatial context to localize novel cell types identified by single cell RNAseq
- Full dataset publicly available for download at NanoString Spatial Organ Atlas

References

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