Spatiotemporal patterns of RNA-binding protein gene expression in the developing embryonic mouse brain

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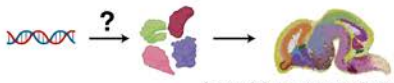
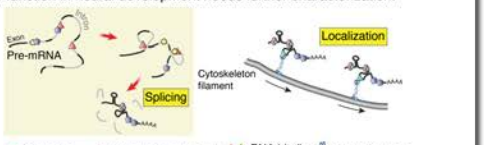
Introduction: Do RNA-binding protein genes display spatial expression patterns in the developing brain?

The brain displays an extraordinary array of cell types and intricate tissue architecture, which arise in part from the diversity of proteins within neural cells. The mechanisms of gene regulation that lead to such diversity remain unclear.



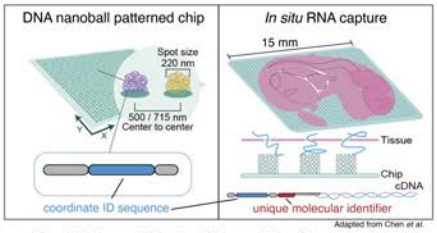
RNA-binding proteins (RBPs) play a role in post-transcriptional gene regulation, but their function in neural development needs further characterization.



We sought to map the expression of RBP genes in the developing mouse brain.

Methods: Stereo-seq provides high-resolution mouse embryonic data for spatial transcriptomics analysis

Stereo-seq is an array-based, *in situ* RNA capture method that provides subcellular resolution of gene expression across a large field of view.[1]

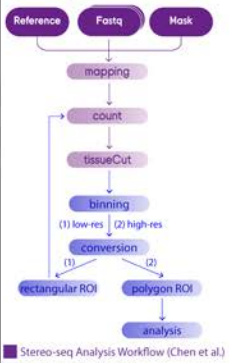


The **Mouse Spatial Transcriptomics Atlas** contains Stereo-seq data for mouse embryos from embryonic days 9.5 through 16.5.[1]

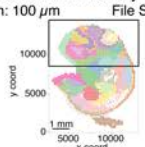
We reprocessed this data at embryonic days 11.5, 12.5, and 14.5 using the Stereo-seq Analysis Workflow[1] and analyzed the data using **Giotto Suite**.[2] We performed Leiden clustering to identify tissues and their subregions, Binary Spatial extract to identify spatially coherent expression of RBPs,[2] and Pearson correlation to identify coexpressed RBPs.

Methods: An adapted pipeline allows for efficient data processing in an anatomical region of interest

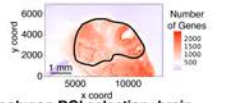
There are several billion reads per embryo slice, so working with the entire dataset at once is unwieldy. We adapted the existing processing pipeline to capture high-resolution data in the brain region.



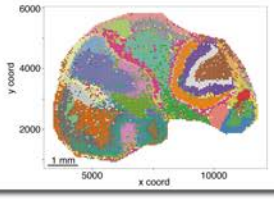
(0) Before rectangular region of interest (ROI) selection: full embryo
Resolution: 100 μm File Size: 500 MB



(1) After rectangular ROI selection: brain
Resolution: 10 μm File Size: 2.66 GB



(2) After polygon ROI selection: brain
Resolution: 10 μm File Size: 1.21 GB

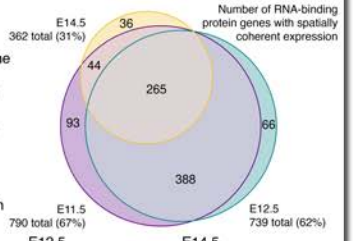


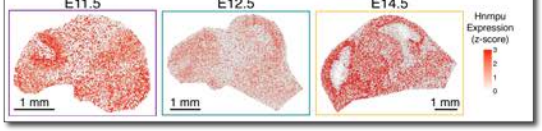
Using the data processed with this method, Leiden clustering on gene expression reveals distinct anatomical regions of the brain. Each color in the plot represents a distinct Leiden cluster.

Results: RNA-binding protein gene expression is spatially coherent

892 out of 1,185 total RNA-binding proteins show spatially coherent gene expression during at least one embryonic day. A spatially coherent gene is more likely than not to be highly expressed in proximal cells.

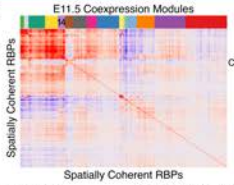
For example, Hnrnpu (heterogeneous nuclear ribonucleoprotein U) shows spatially coherent expression across embryonic time.



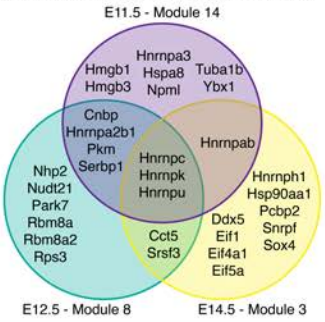


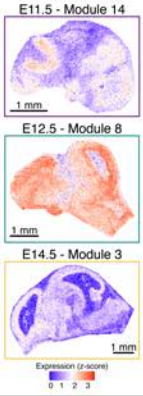
Results: RNA-binding protein gene expression is coordinated across space and time

At each time point, we determined the spatial correlation of the RBP genes with each other. We then grouped the RBPs into 15 modules based on most correlation.



Using the top 15 most correlated RBP genes in each module, we identified a group of modules with high similarity in gene composition and spatial expression pattern across embryonic time.





Conclusions and Future Directions

- There is now a **working, space-efficient pipeline** for analyzing Stereo-seq data.
- **RNA-binding proteins show spatially coherent gene expression** patterns in the developing mouse brain.
- Some RNA-binding protein genes show spatially correlated expression across embryonic time. In the future, we will quantify how their expression levels and locations change over time.
- The RNA-binding proteins identified in this work can be used by other researchers to guide functional studies. For example, we plan to look at the spatial relationships between alternatively spliced transcripts and RNA-binding proteins.

References and Acknowledgements

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