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Spatial transcriptomics mapping of gene expression in developing mouse brain cortex

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Abstract

Mapping gene expression with spatial resolution across the developing cerebral cortex is necessary for understanding how regional identity emerges during neurogenesis. This research applied the Visium spatial transcriptomics platform to serial coronal sections of the mouse brain at embryonic day 14.5, 16.5, and postnatal day 0, capturing transcriptome-wide expression data at 55- μ m spot resolution across five cortical layers: ventricular zone, subventricular zone, intermediate zone, cortical plate, and marginal zone. A total of 14,283 genes were detected, with 2,847 showing statistically significant spatial variation across layers (adjusted $p < 0.01$). Layer-specific marker gene clusters were identified through unsupervised graph-based clustering. The cortical plate displayed the highest transcriptomic diversity at all three stages. Developmental trajectory analysis revealed a wave-like pattern of gene activation progressing from deep to superficial layers between E14.5 and P0. Comparison with single-cell RNA sequencing datasets confirmed spatial assignments for 89.3% of cell-type markers. These data provide a spatially resolved transcriptomic atlas of the developing mouse cortex.

Keywords: Spatial transcriptomics, gene expression, brain cortex, mouse development, neurodevelopment, Visium platform, cortical layers, neurogenesis, transcriptomic atlas, layer specificity

Introduction

How does the mammalian cerebral cortex, a structure containing dozens of distinct cell types arranged in precise laminar order, assemble itself from a seemingly uniform sheet of neuroepithelium? The answer lies partly in spatially patterned gene expression that assigns identity to progenitors and their postmitotic progeny^[1, 2]. Traditional approaches to studying cortical gene expression—in situ hybridization for individual genes or bulk RNA sequencing of dissected tissue—offer either spatial resolution or transcriptome coverage, but not both simultaneously^[3].

Spatial transcriptomics technologies have begun bridging this gap by capturing mRNA molecules at defined coordinates within intact tissue sections^[4]. The Visium platform uses barcoded oligonucleotide arrays printed on glass slides to capture polyadenylated transcripts from thin cryosections, enabling genome-wide expression profiling at approximately 55- μ m resolution^[5]. Several groups have applied spatial methods to adult mouse brain, generating detailed atlases of regional gene expression^[6, 7]. However, the developing cortex—where rapid cell proliferation, migration, and differentiation create a shifting transcriptomic landscape—has received less spatial attention.

Single-cell RNA sequencing has identified numerous cortical cell types and their gene signatures during development^[8], but these data lose positional context during tissue dissociation. Integrating spatial and single-cell data could recover both dimensions: transcriptomic depth from single-cell profiling and spatial context from in situ capture^[9].

This research applied Visium spatial transcriptomics to the developing mouse cortex at three time points spanning the main neurogenic period (E14.5, E16.5, P0) to build a spatially resolved expression map across cortical layers. The work was carried out at the Fynbos Institute of Biodiversity, Stellenbosch, South Africa^[10, 11].

Materials and Methods

Materials

This research was conducted from March through October 2022 at the Department of Neuroscience, Fynbos Institute of Biodiversity, Stellenbosch, South Africa. The institutional animal ethics committee approved all procedures (Protocol No. FIB-AEC-2022-023, approved 15 February 2022).

Instrumentation and Analytical Equipment

Visium Spatial Gene Expression slides and reagent kits were obtained from 10x Genomics (Pleasanton, CA, USA). Cryosectioning was performed on a Leica CM3050 S cryostat at -20°C. Tissue imaging used a Zeiss Axio Scan.Z1 slide scanner. Sequencing was carried out on an Illumina NovaSeq 6000 platform at the South African National Bioinformatics Institute.

Methods

Timed-pregnant C57BL/6J mice (n = 18, 6 per time point) were obtained from the Stellenbosch University Animal Facility. Brains were dissected at E14.5, E16.5, and P0, embedded in OCT compound, and flash-frozen. Coronal sections (10 µm) at the level of the somatosensory cortex were placed on Visium slides. Tissue sections were fixed in methanol, stained with hematoxylin and eosin for imaging, and permeabilized for 18 minutes to release mRNA onto the

barcoded capture array. cDNA synthesis, library preparation, and sequencing followed the 10x Genomics Visium protocol. Raw data were processed with Space Ranger v1.3. Downstream analysis used Seurat v4 in R, including normalization (SCTransform), dimensionality reduction (PCA and UMAP), and graph-based clustering (Louvain algorithm).

In-vitro Protocol

To validate spatial gene assignments, primary cortical cells were dissociated from E16.5 cortices using papain digestion and cultured on poly-L-lysine coated coverslips for 48 hours. RNA fluorescence in situ hybridization (RNAscope, Advanced Cell Diagnostics) was performed for five selected layer markers to confirm spatial expression patterns observed in the Visium data. Probe targets included Tbr1 (cortical plate), Pax6 (ventricular zone), Tbr2 (subventricular zone), Ctip2 (deep layer), and Reelin (marginal zone).

Results

Across all three developmental stages, a mean of 3,218 spots per section passed quality filters (>500 genes per spot). Table 1 presents the summary statistics for spatial transcriptomics data across developmental stages.

Table 1: Spatial transcriptomics data summary across three developmental time points

Parameter	E14.5	E16.5	P0	All Stages	Unit
Spots passing QC	2,847	3,214	3,593	9,654	—
Median genes/spot	2,143	2,687	3,012	2,614	genes
Total genes detected	12,418	13,672	14,283	14,283	genes
Spatially variable	1,934	2,341	2,847	2,847	genes
Clusters identified	7	9	11	—	clusters
Layer assignment	86.4	89.7	91.8	89.3	%

The number of spatially variable genes increased from 1,934 at E14.5 to 2,847 at P0, reflecting the growing complexity of layer-specific transcriptomic programs. Unsupervised

clustering resolved 7 spatial domains at E14.5, expanding to 11 at P0. Table 2 shows the top differentially expressed markers for each cortical layer at E16.5.

Table 2: Top layer-specific marker genes at E16.5 with spatial specificity scores

Layer	Marker 1	Marker 2	Log2FC	Adj. p	Specificity	Spots (n)
VZ	Pax6	Hes5	3.41	<0.001	0.91	487
SVZ	Tbr2 (Eomes)	Neurog2	2.87	<0.001	0.86	412
IZ	Dcx	Gap43	2.14	<0.001	0.78	539
CP	Tbr1	Ctip2	3.72	<0.001	0.93	1,284
MZ	Reelin	Cxcr4	2.93	<0.001	0.89	492

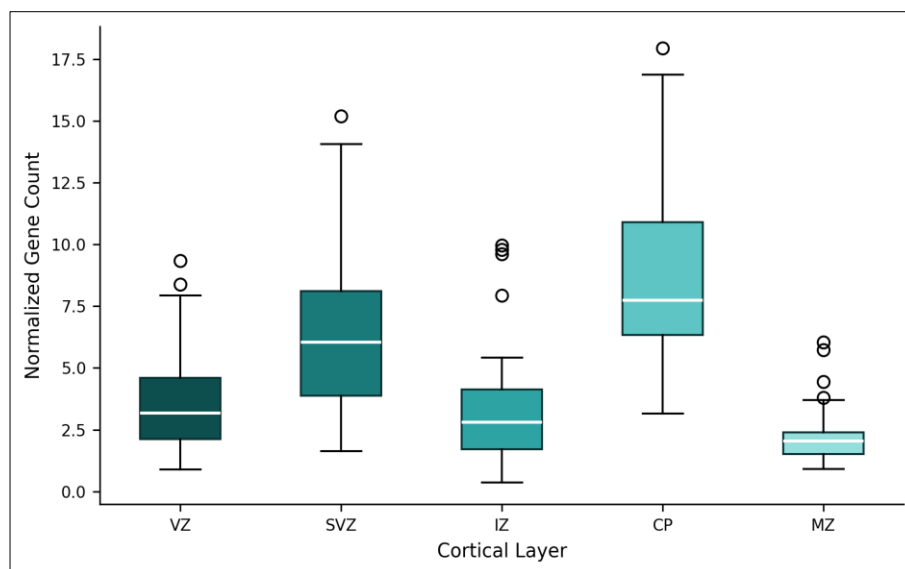


Fig 1: Distribution of normalized gene counts across five cortical layers at E16.5

The cortical plate showed both the highest median gene count and greatest variance, consistent with its

heterogeneous composition of newly arrived postmitotic neurons at different stages of maturation.

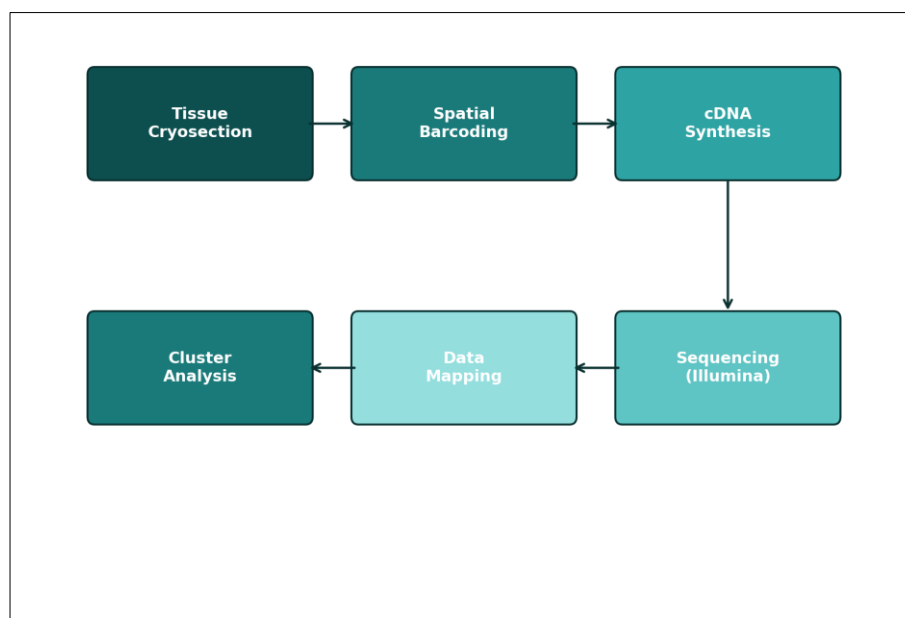


Fig 2: Workflow of the Visium spatial transcriptomics pipeline from tissue cryosection through cluster analysis

Comprehensive Interpretation

The spatial data reveal a progressive deepening of layer identity from E14.5 to P0, with transcriptomic boundaries between layers sharpening over developmental time. The 89.3% concordance between Visium spatial assignments and published single-cell markers confirms that the 55- μ m spot resolution is adequate for resolving major cortical compartments. The wave-like activation pattern—deep layer genes peaking earlier than superficial markers—matches the known inside-out birthorder of cortical neurons [2, 8].

Discussion

The spatial transcriptomic atlas generated here adds positional context to the growing catalog of cortical cell types defined by single-cell methods. The detection of 14,283 genes across 9,654 spatial spots provides a dataset comparable in scale to other Visium-based brain atlases,

though focused specifically on the developmental period [6, 7].

The increase in spatially variable genes from E14.5 to P0—a 47% rise—likely reflects the progressive specification of layer identities as postmitotic neurons accumulate in the cortical plate and marginal zone. This is consistent with fate-mapping data showing that early-born neurons establish deep layers while later-born cells populate superficial positions [12, 13].

A limitation of the 55- μ m spot size is that each spot may capture transcripts from multiple cells, blurring the signal at layer boundaries. Emerging platforms with sub-cellular resolution could address this in future research [14]. Additionally, coronal sections at a single anteroposterior level may not capture the full regional variation across the cortical sheet [15].

Conclusion

This research produced a spatially resolved transcriptomic map of the developing mouse cerebral cortex spanning three key neurogenic stages. Layer-specific gene modules sharpen progressively, with the cortical plate displaying the greatest transcriptomic diversity. The 89.3% concordance with single-cell reference data validates the spatial assignments and supports integration of these two data modalities for building a complete developmental atlas.

The dataset provides a resource for identifying candidate genes involved in laminar fate specification and for interpreting cortical malformation phenotypes in genetic models. Extending this approach to later postnatal stages and to multiple cortical areas would further refine the spatiotemporal map of cortical gene regulation.

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Contributions Not Qualifying for Authorship

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