Spatial Gene Expression: Resolving the mammalian brain by combining anatomical and sequencing data

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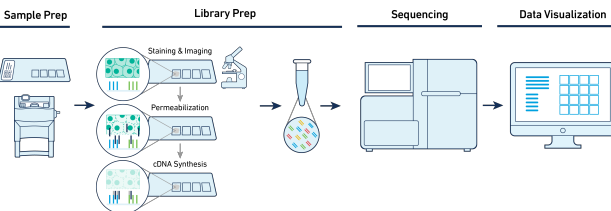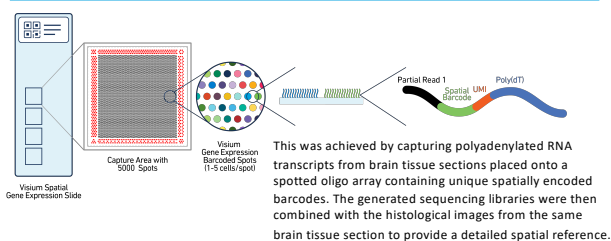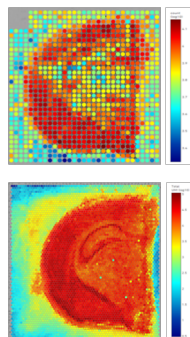
1. Introduction

Identifying individual cells and their genetic makeup are critical for understanding their roles in how the central nervous system (CNS) physiologically functions, develops, and organizes; as well as how these modalities are altered in diseased states. Traditional methods lack either the cellular resolution and/or throughput necessary to fully compare the different cell types that exists within the mammalian CNS. Spatial transcriptomics technology addressed these limitations by integrating both histological and sequencing data[1]. Here we significantly improved the spotted oligo array technology that increases tissue coverage and spatial resolution by reducing spot size, and increasing spot number and packing density. Through advancements in the biochemistry, we exhibited substantially increased sensitivity while simultaneously reducing experiment duration and expanding tissue compatibilities. These improvements allow for an unbiased clustering of cell type that reliably correlates with the neuroanatomy of both rodents and human specimens. Furthermore, application of the technology could provide a powerful tool for understanding neurological diseases and discovery of potentially new therapeutic/diagnostic targets.

## 2. Unbiased spatial gene expression methodology overview

This was achieved by capturing polyadenylated RNA transcripts from brain tissue sections placed onto a spotted oligo array containing unique spatially encoded barcodes. The generated sequencing libraries were then combined with the histological images from the same brain tissue section to provide a detailed spatial reference.

The Spatial Gene Expression workflow:

1. Fresh frozen tissues are embedded in OCT
2. Sectioned tissues are placed on the capture areas of the library prep slides
3. Samples are stained with H&E and imaged
4. Tissues are permeabilized to capture transcripts
5. Following reverse transcription, samples undergo second strand synthesis and cDNA is generated
6. cDNA is amplified
7. Prepare libraries for 3' gene expression
8. Sequence
9. Analyze data
10. Visualize data

Please contact info@10xgenomics.com for more information.
https://www.10xgenomics.com/spatial-transcriptomics/

3. Spatial gene expression characterization across the mouse hippocampal and various brain regions.

A) Spatial clustering of the hippocampus and neighboring areas using the spatial gene expression technology (inset - H&E reference). Spatial clustering and t-SNE plots identify brain regions in an unbiased manner. Restriction of gene expression in specific hippocampal areas. B) Spatial clustering and gene expression anteriorly within the olfactory bulb. C) Spatial clustering and gene expression posteriorly within the cerebellum. (Scale bars = 1.0 mm)
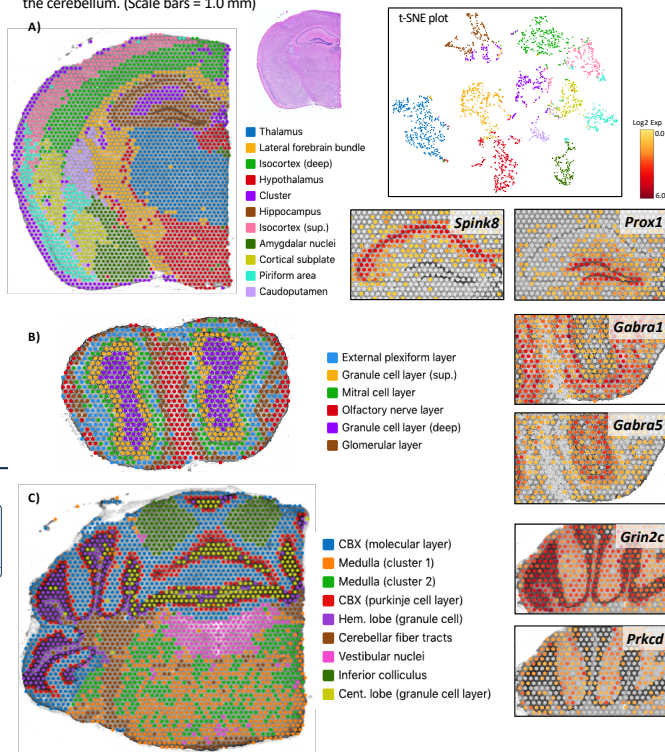

4. Spatial clustering and *Gria1* expression across the sagittal mouse brain.

A) Spatially-resolved clustering of the anterior and posterior regions of the mouse brain split across two capture areas. Examination of *Gria1* (Glutamate Ionotropic Receptor AMPA type Subunit 1) expression of across multiple regions of the brain.
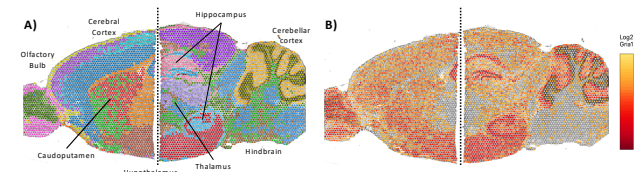

5. Spatial data correlates with single nuclear RNA-seq.

A) Serial sections between different capture areas demonstrate strong correlation with the examination of 10,000 randomly selected genes. B) Obtained spatial sequencing data correlate with single-nuclear 3' RNA sequencing from the consecutive sections collected the same brain sample.
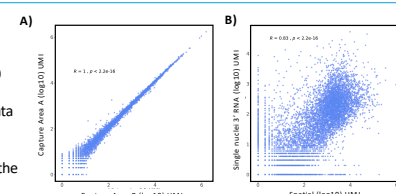

## 6. Compatibility with rat and human neuronal tissue.

A) Spatially-resolved clustering of the Sprague-Daley rat olfactory bulb with a demonstration of *Gabra1* expression corresponding to the external plexiform layer (inset). B) Spatially-resolved clustering of cerebellar tissue from a female patient (BioIVT: Asterand; Case ID 9917; Specimen ID 1215079F)[2] with a demonstration of *Neurod1* expression corresponding to the granule cell layer (inset).
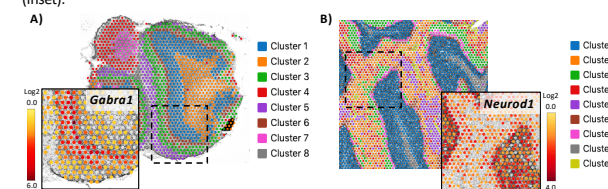
7. Application of spatial gene expression on the APPSWE [Tg2576] model of familial Alzheimer's disease
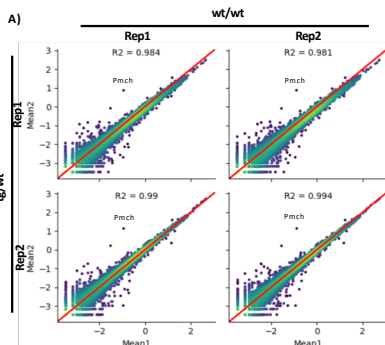
Proof of concept application of spatial gene expression technology to 12-month-old male APPSWE [Tg2576] mouse model of familial Alzheimer's Disease[3] (Taconic Biosciences)[4]. A) Differential gene expression between wt/wt and tg/wt mice highlight elevated expression of pro-melanin concentrating hormone (*Pmch*). B) Examination of neurogranin (*Nrgn*) expression demonstrate similar spatial pattern between wt/wt and tg/wt mice. However, spatial examination of *Pmch* demonstrated increased expression within the hypothalamic region, as well as other areas within tg/wt mice.
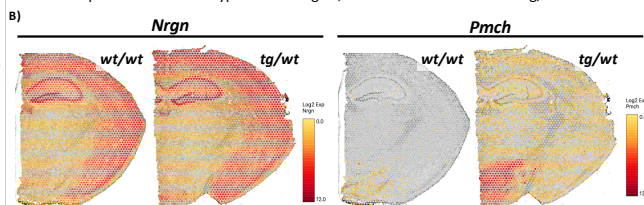
8. Conclusions

This technology demonstrated:

- The ability to examine histological and transcriptome profiles from the same tissue section at a much higher resolution, better sensitivity, and shorter time.
- Obtain unbiased and high-throughput gene expression analysis for intact tissue sections across different brain regions from both rodent specimens and human patients.
- Generate spatial clustering that reliably correlates with the neuroanatomy.
- Demonstrate the ability to discover novel targets and/or pathways with unbiased analysis.

9. References

1. Science. 2016 Jul 1;353(6294):78-82.
2. BioIVT: Asterand (https://www.bioivt.com/)
3. Science. 1996 Oct 4;274(5284):99-102.
4. Taconic Biosciences (https://www.taconic.com/)