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 capture the different cell types that exists within the mammalian CNS. Spatial transcriptomics technology addressed these limitations by integrating both histological and sequencing data. 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 improved the spotted oligo array technology that increases tissue coverage and spatial cellular resolution and/or throughput necessary to fully compare the different cell types that

2. Unbiased spatial gene expression methodology overview

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 Gabra2 expression corresponding to the external plexiform layer (inset). B) Spatially-resolved clustering of cerebellar tissue from a female patient (BioVit: Asterand, Case ID 9917; Specimen ID 1215079F) 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 Disease1 (Taconic Biosciences®). A) Differential gene expression between wt/wt and tg/tg mice highlight elevated expression of pro-melanin concentrating hormone (Pmch). B) Examination of neuregulin (Nrgn) expression demonstrate similar spatial pattern between wt/wt and tg/tg mice. However, spatial examination of Pmch demonstrated increased expression within the hypothalamic region, as well as other areas within tg/tg 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 nervous system.
- Demonstrate the ability to discover novel targets and/or pathways with unbiased analysis.

9. References
4. Taconic Biosciences (https://www.taconic.com/)