Technical Note
Evercode Whole Transcriptome

Comparison of Evercode™ WT v3 and Chromium™ GEM-X Single Cell 3′ Kit v4 in Mouse Brain Nuclei

Introduction

Here we present the results of a head-to-head evaluation of single cell RNA-seq technologies between droplet-based microfluidics (10x Genomics™ Chromium GEM-X Single Cell 3′ Kit v4) and combinatorial barcoding (Parse Biosciences Evercode WT v3). The comparison was performed using mouse brain nuclei, a complex and relevant sample type in many areas of scientific discovery.

Comparison Highlights

- Head-to-head sensitivity comparison shows a superior gene detection with Evercode WT v3.
- Cell type proportions are equivalently represented.
- Evaluation of differentially expressed genes reveals that Evercode detects twice as many differentially expressed genes.

EXPERIMENTAL DESIGN

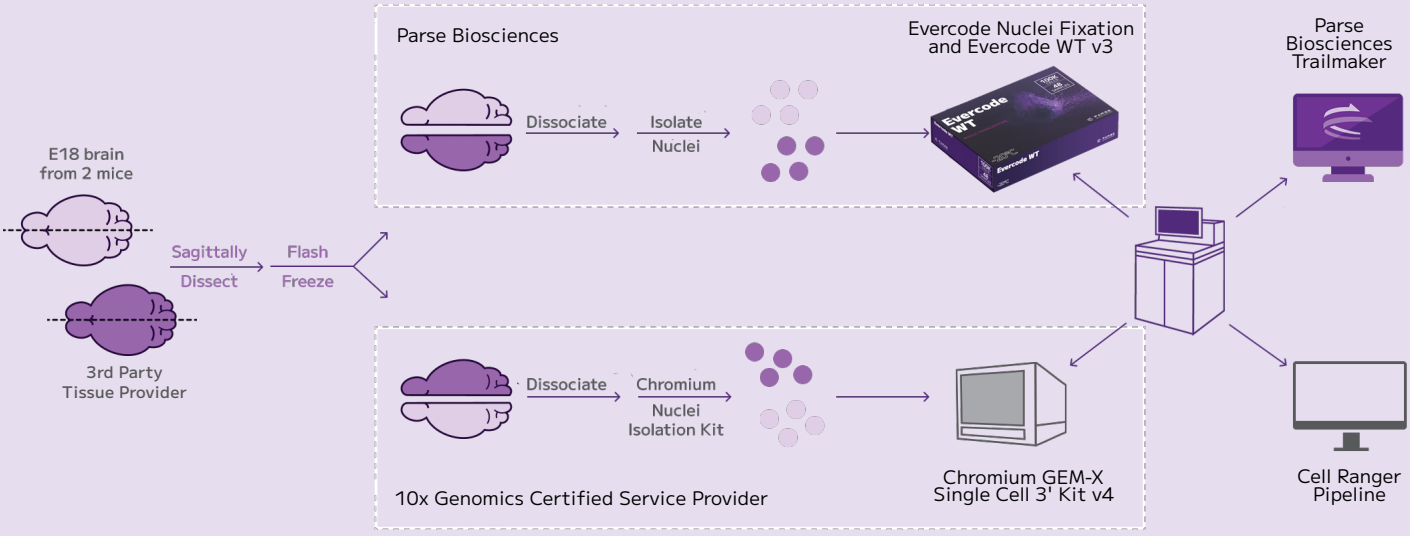

Figure 1. Experimental Design. Two embryonic mouse brain samples were collected, sagittally dissected into 2 halves, and flash frozen by a third-party tissue vendor. Half of each brain was shipped to a 10x Genomics certified service provider to isolate nuclei and create sequencing libraries. The other halves of each brain were processed by Parse Biosciences for nuclei isolation, fixation, and library preparation. Sequencing libraries from each technology were sequenced by a third-party. The sequencing data were processed by each manufacturer's data analysis pipeline, after which they can be integrated in Trailmaker for downstream analysis and visualization.

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Methods

Sample Collection

Brain samples from two E18 embryonic C57/Bl6 mice were collected, microsurgically dissected, and had the meninges removed by a third-party tissue provider. This was done to ensure the same starting quality for each sample to accurately measure the technologies. The left and right hemispheres of each brain were separated prior to flash freezing for shipment to the testing laboratories.

10x Genomics Chromium GEM-X 3' v4

The right hemisphere of Mouse 1 and left hemisphere of Mouse 2 were shipped to a 10x Genomics Certified Service Provider for sample and library preparation. Nuclei were isolated with the Chromium Nuclei Isolation Kit. With a target of 20,000 nuclei per sample, a Chromium GEM-X Single Cell 3' GEM Library & Gel Bead Kit v4 was used to partition and prepare sequencing libraries. All activities were performed according to the procedures of the Certified Service Provider.

Parse Biosciences Evercode WT v3

Nuclei were isolated from the left hemisphere of Mouse 1 and right hemisphere of Mouse 2 using a dounce homogenizer. The nuclei were then fixed with Evercode Nuclei Fixation v3. Whole transcriptome sequencing libraries were prepared with a target of 10,000 nuclei per sample using Evercode WT v3.

Sequencing and Data Analysis

10x Genomics libraries were sequenced on an Illumina© Novaseq™ X by the certified service provider, and the Parse Biosciences libraries were sequenced on the same instrument model. The 10x Genomics data were processed with Cell Ranger™ v8.0.1 with intron mode enabled, and the Parse Biosciences data were processed with the Parse Biosciences analysis pipeline v1.3.0.

Integration and Cell Type Annotation

All libraries were downsampled to the same mean reads per cell. The Parse Evercode WT v3 and 10x Genomics GEM-X 3' v4 datasets were integrated with Harmony using Trailmaker, and marker genes were mapped to the mouse brain wheel reference (http://mousebrain.org/wheel/) for cell type annotation. Differential expression analysis was performed for all cell types using the presto implementation of the Wilcoxon rank sum test, counting the up-regulated genes with >0.25 log fold change and adjusted $p<0.001$.

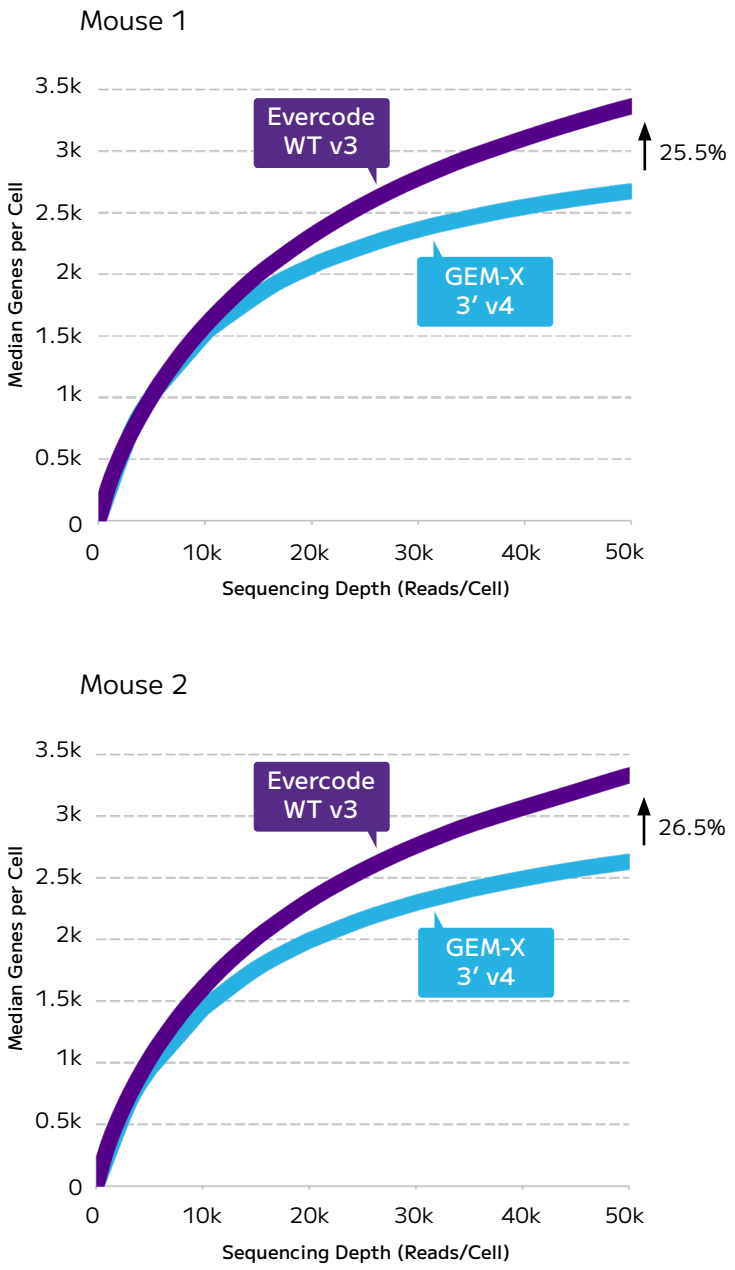

Figure 2. Gene Detection. Median genes detected per nuclei across different sequencing depths for mouse brain 1 (top) and mouse brain 2 (bottom). Each mouse brain was sagitally dissected into two halves and sent to different laboratories for processing with either Evercode WT v3 or Chromium GEM-X 3′ v4 technologies and processed by their respective data analysis pipelines.

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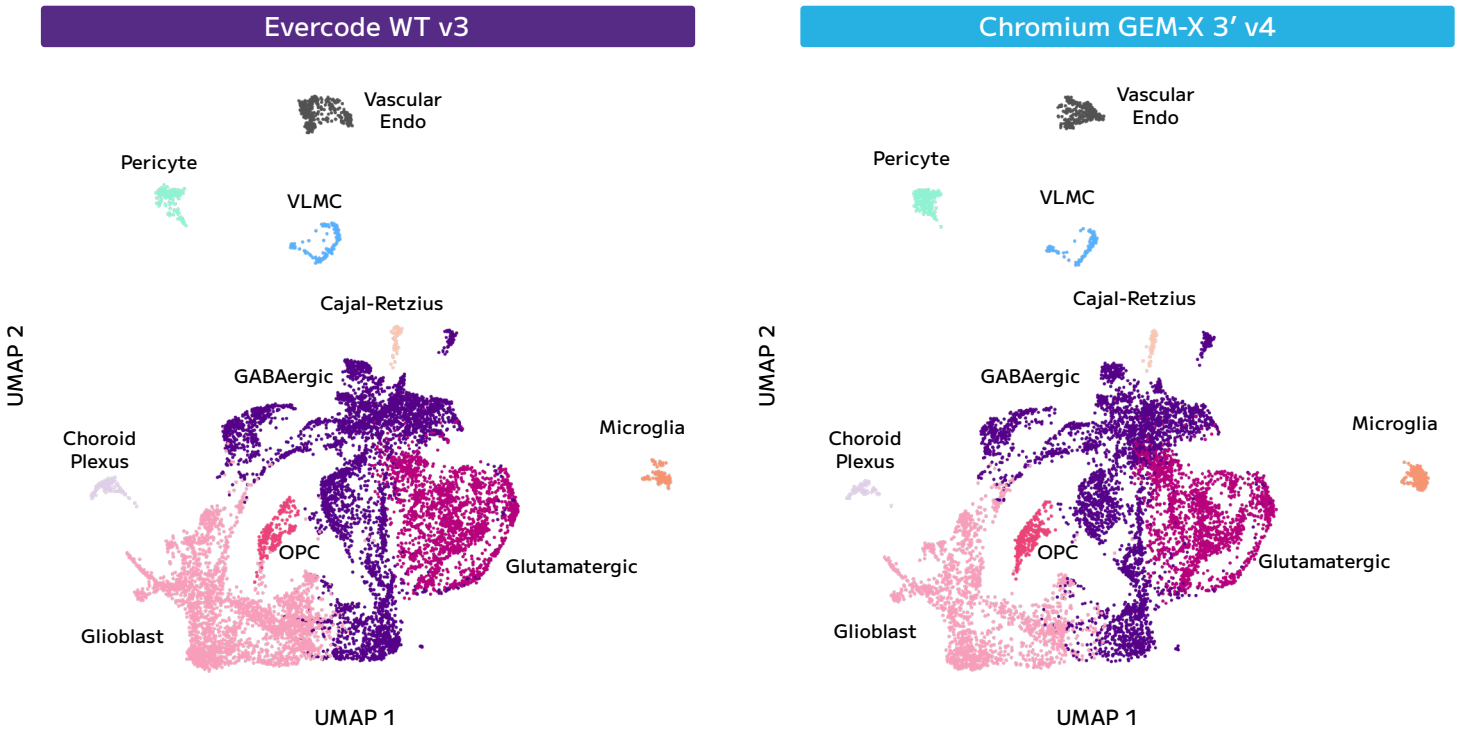
Consistent Gene Expression and Proportions of Cell Types

A. Clustering of integrated Evercode WT v3 and Chromium GEM-X 3′ v4 data



B. Comparison of relative abundance of cell types

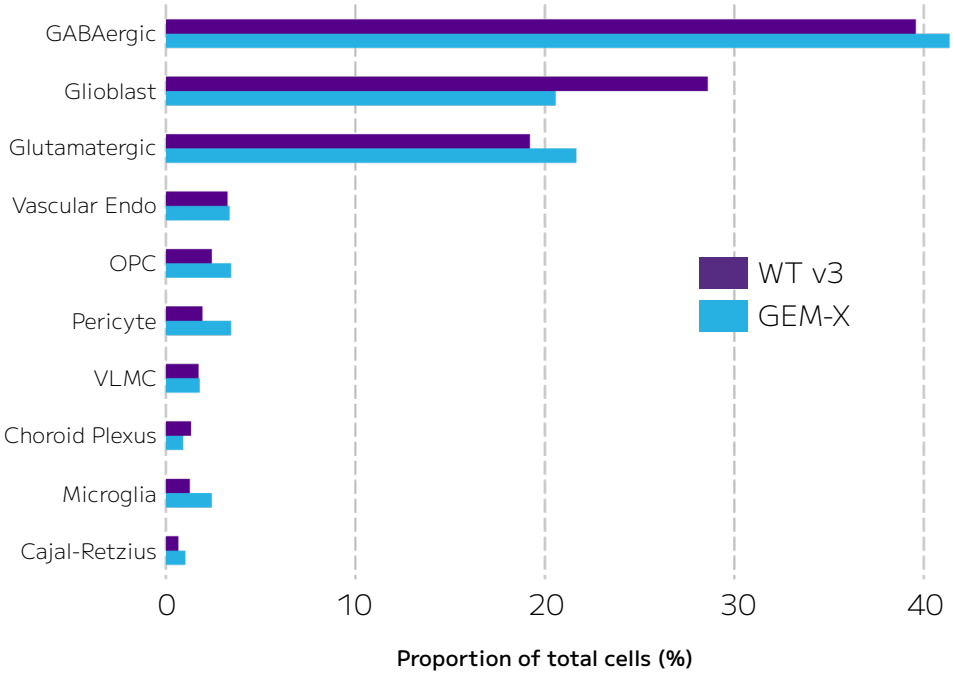
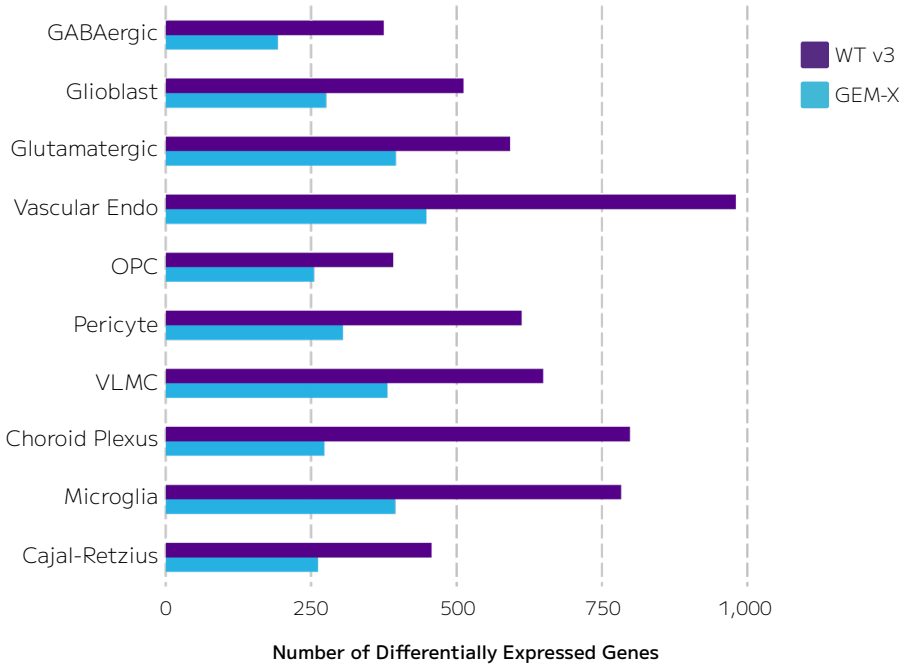


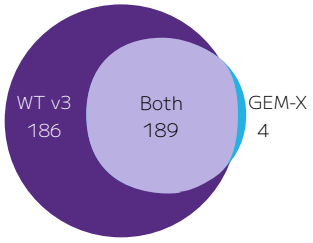
Figure 3. Gene Expression Profile Comparison.
(A) 10,000 nuclei from Evercode WT v3 and 10,000 nuclei from Chromium GEM-X 3' v4 were integrated, clustered, annotated with Trailmaker, and visualized separately in annotated UMAPs. This dataset is freely available for exploration in the Trailmaker data repository (https://app.trailmaker.parsebiosciences.com/repository).
(B) A comparison of relative abundance of the major cell types (presented as % of total cells) was performed to confirm concordance of expression between the technologies.

Increased Detection of Cell Markers

A. Differentially expressed genes that define cell types



B. GABAergic Neurons



C. Cajal-Retzius

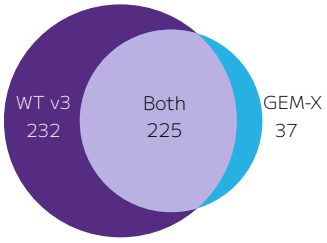


Figure 4. Comparison of Differentially Expressed Genes. (A) The number of differentially expressed genes for each major cell type, ordered by abundance of the cell type. The uniqueness of differentially expressed genes in the highest (B, GABAergic Neurons) and lowest (C, Cajal-Retzius) abundant cell types were further investigated. Differentially expressed genes unique to Evercode WT v3 in purple, unique to Chromium GEM-X 3′ v4 in blue, and common to both technologies are shown at the intersection.

Results

Sensitivity is 26% Higher with Evercode
Increased sensitivity enables better detection of lowly expressed genes, resulting in more comprehensive annotation of cell types. In this comparison, the Evercode WT v3 detected an average of 26% more genes than the Chromium GEM-X 3′ v4 at 50,000 reads/cell (Figure 2).

Cell Proportions are Concordant
Integration of the data from Evercode WT v3 and Chromium GEM-X 3′ v4 resulted in highly concordant clustering and cell proportions (Figure 3), indicating both technologies result in unbiased capture of cell types.

More Differentially Expressed Genes Detected
Investigation of the number of differentially expressed genes for each cell type showed that Evercode WT v3 consistently detected more differentially expressed genes than Chromium GEM-X 3′ v4 across all cell types (Figure 4). For GABAergic Neurons, the most abundant cell type, the Parse Biosciences data resulted in 94% more differentially expressed genes compared to 10x Genomics. For Cajal-Retzius, the least abundant cell type, Parse Biosciences data resulted in 74% more differentially expressed genes.

Conclusion

This evaluation compared two of the leading single cell gene expression products available to researchers, the 10x Genomics Chromium GEM-X Single Cell 3′ Kit v4 and Parse Biosciences Evercode WT v3.

Evercode WT v3 outperformed the Chromium GEM-X 3′ v4 in gene detection at all sequencing depths. Both technologies recovered similar proportions of cell types. Evercode WT v3 detected substantially higher numbers of differentially expressed genes across all cell types. When compared to the Chromium GEM-X 3′ v4, these data show Evercode WT v3 yielded higher sensitivity and more information per cell.

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