Comparison of Evercode™ WT v2 and Chromium™ Next GEM Single Cell 3' Kit v3.1 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 Next GEM Single Cell 3' Kit v3.1) and combinatorial barcoding (Parse Biosciences Evercode WT v2). 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 marked contrast in gene detection.

- Cell type proportions are equivalently represented.

- Evaluation of differentially expressed genes reveals a disparity in the information attainable with each technology.
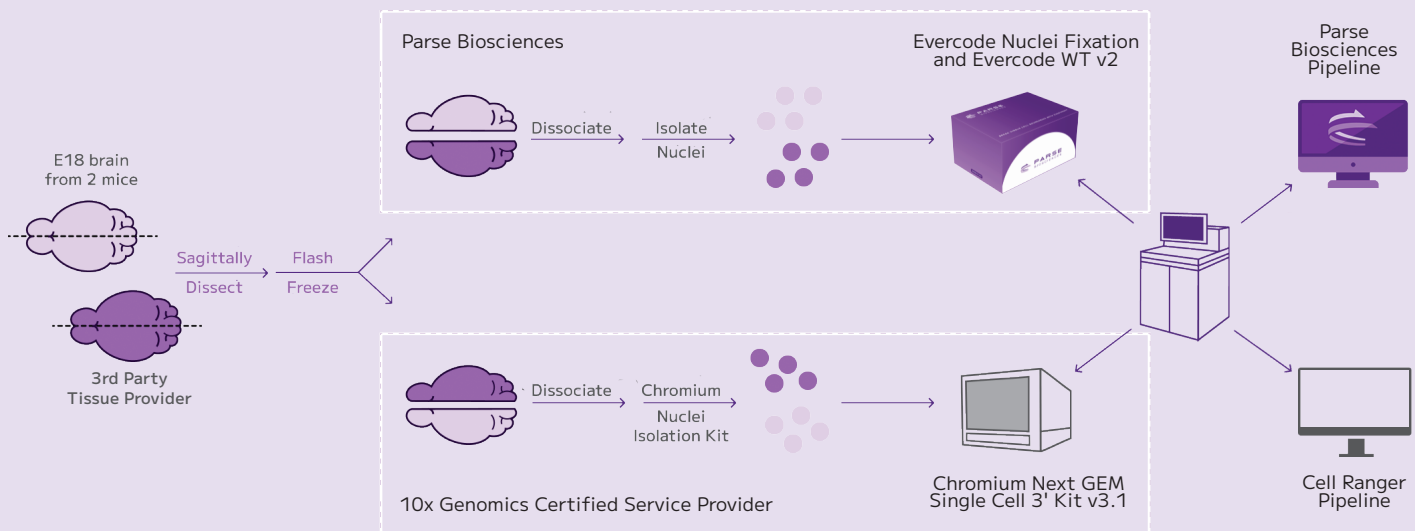
EXPERIMENTAL DESIGN

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 analyzed by each manufacturer's data analysis pipeline.
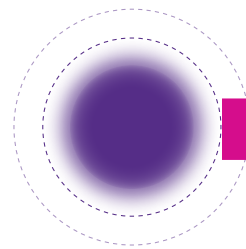
Methods

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. The left
and right hemispheres of each brain were separated prior to flash
freezing for shipment to the testing laboratories.

10x Genomics Chromium Next GEM 3' v3.1

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 10,000 nuclei
per sample, a Chromium Next GEM Single Cell 3' GEM Library &
Gel Bead Kit v3.1 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 v2

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 v2. Whole
transcriptome sequencing libraries were prepared with a target of
10,000 nuclei per sample using Evercode WT v2.

Sequencing and Data Analysis

Both the 10x Genomics and the Parse Biosciences libraries were
sequenced on an Illumina© Novaseq™ 6000 by the same
third-party service provider. The 10x Genomics data were analyzed
with Cell RangerTM v7.0.1 with intron mode enabled, and the
Parse Biosciences data were analyzed with the Parse Biosciences
analysis pipeline v0.9.6.

Integration and Cell Type Annotation

All libraries were downsampled to the same mean reads per cell.
The Evercode WT v2 and 10x Genomics 3' v3.1 datasets were
integrated with the Seurat fast integration method (rPCA). The
Atlas of the Developing Mouse Brain from the Linnarsson Lab
(available from http://linnarssonlab.org/) was used as a reference
for mapping and annotation. To obtain the differentially expressed
(DE) genes, the Seurat function FindAllMarkers was run on
the results from each platform and up-regulated genes with an
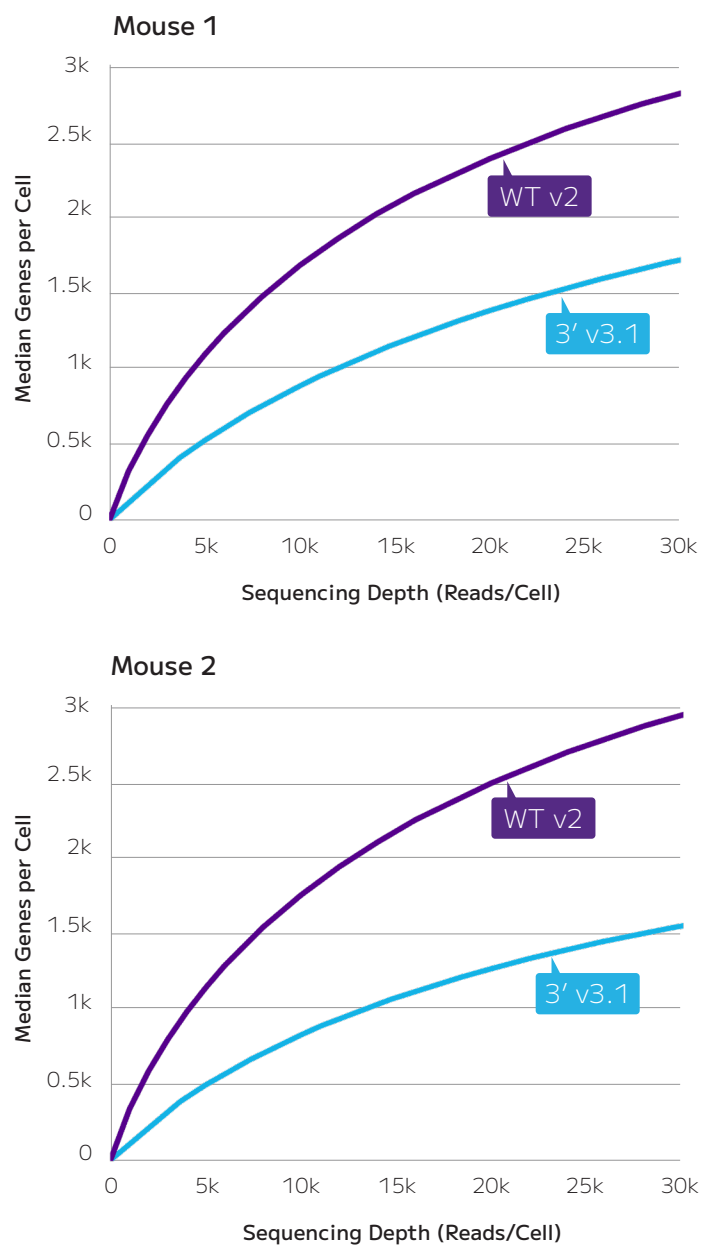adjusted $p<0.001$ were counted.

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 v2 or 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 technologies and analyzed by their respective data analysis pipelines.

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Consistent Gene Expression and Proportions of Cell Types

A. Clustering of integrated Evercode WT v2 and Chromium Next GEM Single Cell Kit v3.1 data

Evercode WT v2

Chromium Next GEM Single Cell 3' v3.1
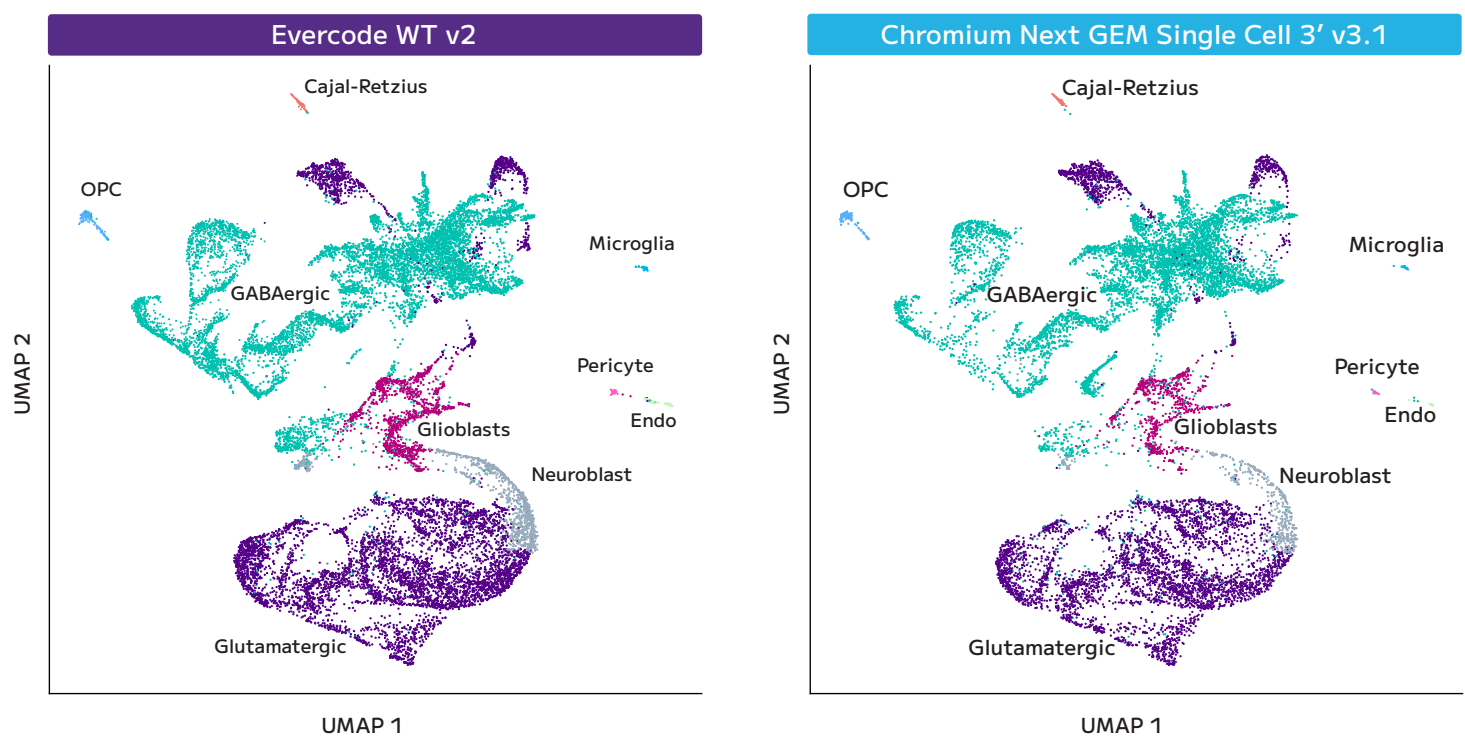
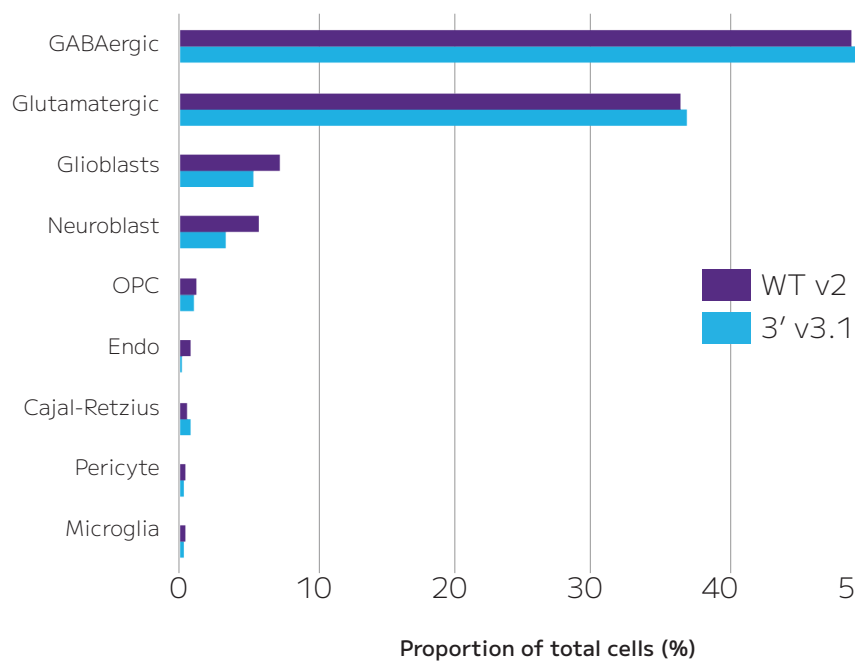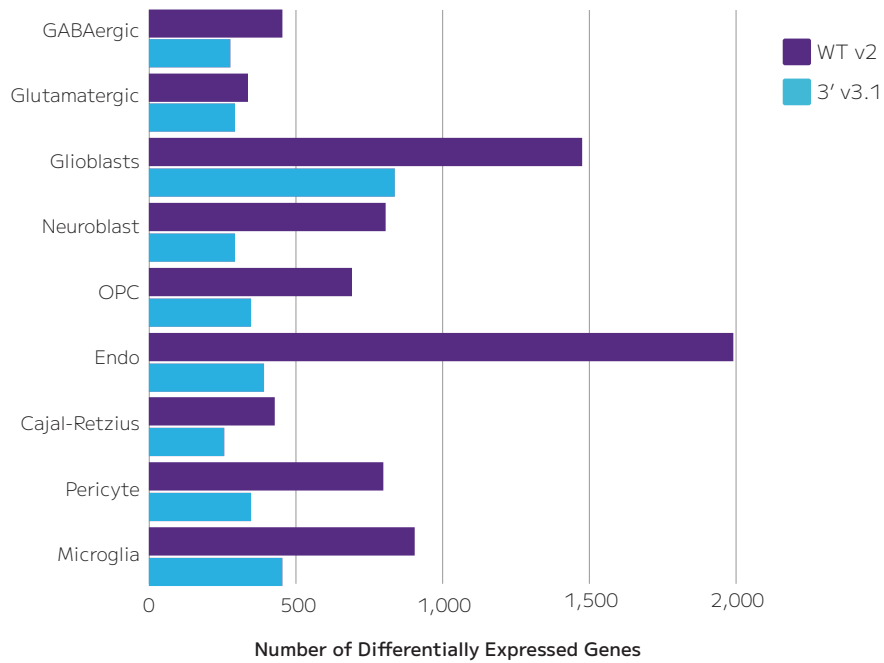## B. Comparison of relative abundance of cell types
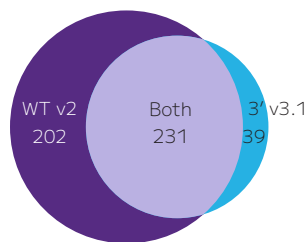

Figure 3. Gene Expression Profile Comparison.
(A) 17,339 nuclei from Evercode WT v2 and 12,967 nuclei from Chromium Next GEM Single Cell 3' Kit v3.1 were integrated, clustered, annotated with Seurat, and visualized separately in annotated UMAPs. (B) A comparison of relative abundance of the major cell 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

Number of Differentially Expressed Genes

Legend: WT v2 (purple), 3' v3.1 (blue)

Cell types: GABAergic, Glutamatergic, Glioblasts, Neuroblast, OPC, Endo, Cajal-Retzius, Pericyte, Microglia

B. GABAergic Neurons

WT v2: 202 | Both: 231 | 3' v3.1: 39

C. Microglia

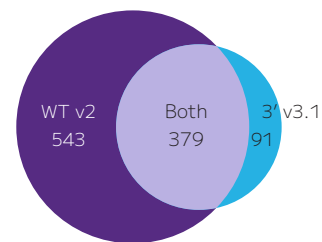WT v2: 543 | Both: 379 | 3' v3.1: 91

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, Microglia) abundant cell types were further investigated. Differentially expressed genes unique to Evercode WT v2 in purple, unique to Chromium Next GEM Single Cell 3' Kit v3.1 in blue, and common to both technologies are shown at the intersection.

Results

Sensitivity
Increased sensitivity enables better detection of lowly expressed genes, resulting in more comprehensive annotation of cell types. In this comparison, the Evercode WT v2 detected an average of 84% more genes than the Chromium Next GEM Single Cell 3' Kit v3.1 at 20,000 reads/cell (Figure 2).

Cell Proportions and Differential Expression
Integration of the data from Evercode WT v2 and Next Gem 3' v3.1 resulted in highly concordant clustering and cell proportions (Figure 3), indicating both technologies result in unbiased capture of cell types.

Investigation of the number of differentially expressed genes for each cell type showed that Evercode v2 consistently detected more DE genes than Chromium Next GEM 3' v3.1 across all cell types (Figure 4). For GABAergic Neurons, the most abundant cell type, the Parse Biosciences data resulted in 60% more differentially expressed genes compared to 10x Genomics. For Microglia, the least abundant cell type, Parse Biosciences data resulted in 96% more differentially expressed genes.
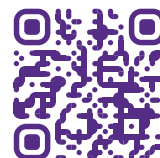
Conclusion

An evaluation compared two of the leading single cell gene expression products available to researchers, the 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 and the Parse Biosciences Evercode WT v2.

Evercode WT v2 outperformed the Chromium Next GEM Single Cell 3' Kit v3.1 in gene detection at all sequencing depths. Both technologies recovered similar proportions of cell types. Evercode WT v2 detected substantially higher numbers of differentially expressed genes across all cell types. When compared to the Chromium Next GEM 3' v3.1, these data show Evercode WT v2 yielded higher sensitivity and more information per cell.

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