Parse BIOSCIENCES

Single Cell 3.0

Scalable Single Cell Sequencing, No Instrument Required

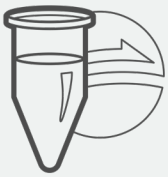
EASY TO GET STARTED | **FLEXIBLE AND SCALABLE** | **UNMATCHED SENSITIVITY**

Use Innovative Technology in Any Lab

SIMPLE WORKFLOW

An instrument-free workflow takes isolated cells or nuclei to biological insights

1 Fixation of cells or nuclei



2 Barcoding and Library Prep

3 Sequencing

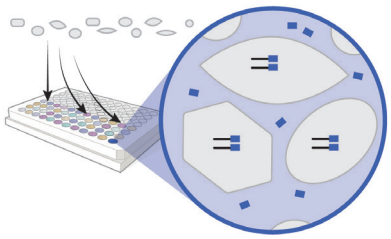
4 Data Analysis

NO INSTRUMENT REQUIRED

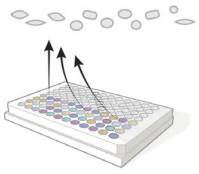
Evercode™ split pool combinatorial barcoding technology converts each cell or nucleus into an individual reaction compartment. This approach steps away from the limitations of hardware-based offerings for a more elegant solution to single cell RNA sequencing (scRNA-seq) and single nuclei RNA sequencing (snRNA-seq).

1 Reverse Transcription

Split | Fixed cells/nuclei are distributed into wells, and the first sample-specific barcodes are added by in-cell reverse transcription.

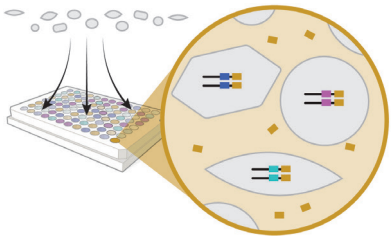


2 Pool | All the cells are pooled together.

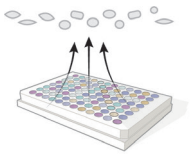


3 Ligation

Split | The pooled cells are distributed across a plate, and an in-cell ligation adds the second barcode.

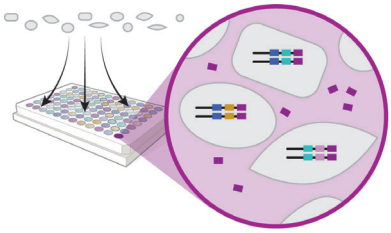


4 Pool | All the cells are pooled together.

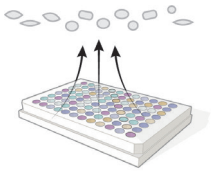


5 Ligation

Split | The pooled cells are again distributed across a plate, and a third barcode is added via in-cell ligation reaction.

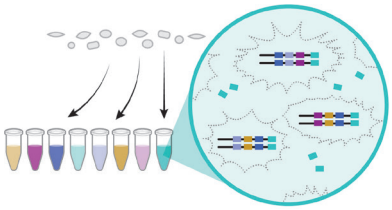


6 Pool | All the cells are pooled together.



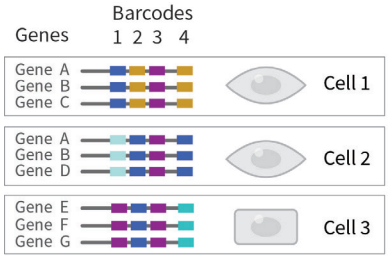
7 Lysis and Library Prep

Split | The pooled cells are distributed across several sublibraries then lysed. The fourth barcode is added via PCR.



8 Sequencing and Analysis

Each transcript is assigned to a single cell based on a unique combination of barcodes.



SINGLE CELL 3.0 ENABLES ANY EXPERIMENT IN ANY LAB

Previous generations of scRNA-seq empowered researchers to explore biological understanding in new ways. Parse Biosciences toppled barriers to its wider adoption with the introduction of Evercode combinatorial barcoding technology by eliminating the need for expensive hardware and enabling unprecedented throughput. This technology delivers sensitive gene and cell type detection across different sample types.

Single Cell 1.0	**Single Cell 2.0**	**Single Cell 3.0**
Individual Cells in Wells	Droplet-based	Combinatorial Barcoding
10 - 100's of cells	1,000 - 10,00's of cells	10,000 - Millions of cells

SENSITIVE AND ACCURATE GENE AND CELL TYPE DETECTION

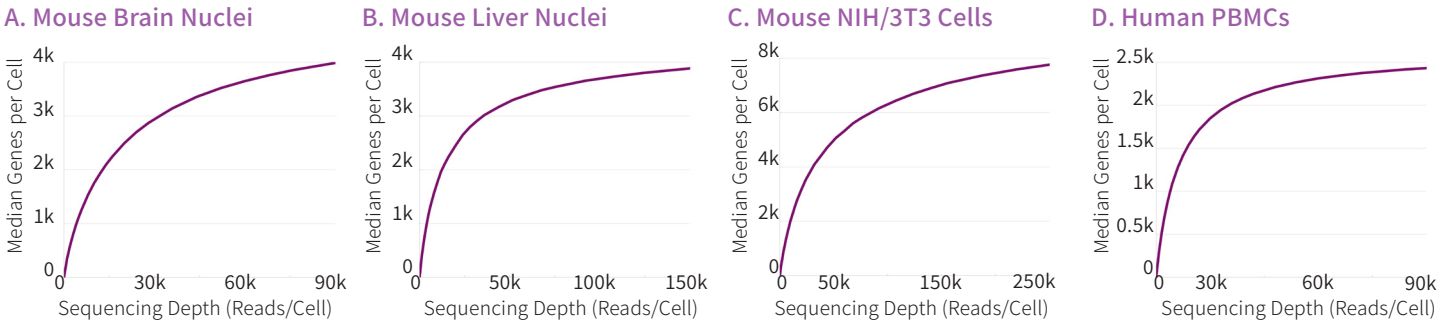


Figure 1. Gene Sensitivity Across Multiple Sample Types. Cells and nuclei from multiple sample types were prepared with Evercode WT v2 and sequenced to saturation in (A) mouse brain nuclei, (B) mouse liver nuclei, (C) the mouse fibroblast cell line NIH/3T3, and (D) human PBMC.

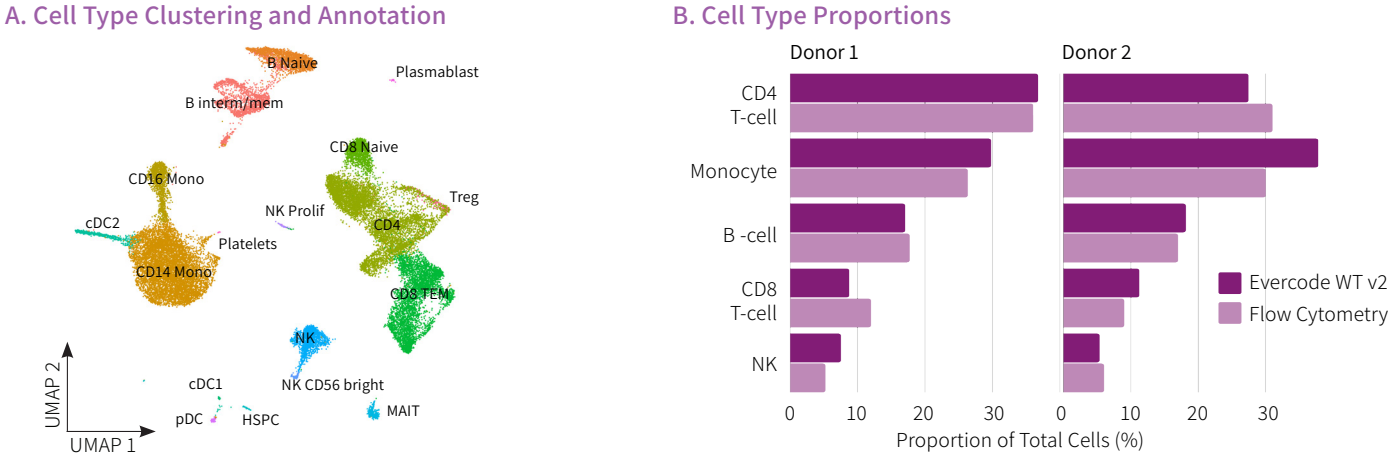


Figure 2. Cell Type Detection in PBMC Samples. Human PBMC samples from 4 healthy donors were fixed and processed with Evercode Cell Fixation and WT v2 kits. (A) Sequencing data was processed with the Parse Biosciences pipeline, integrated, co-clustered, and annotated. (B) Comparison of cell type proportions from two donors demonstrates the accuracy of Evercode scRNA-seq data relative to gold standard flow cytometry data.

BIOINFORMATICS ANALYSIS INCLUDED

The Parse Biosciences pipeline can run locally on your own computer or in the cloud using the DNAnexus® platform. The pipeline produces standard output files (including gene-cell count matrix compatible with third-party analysis tools like Seurat and Scanpy) and an interactive HTML report that enables exploration of cell type clustering and gene expression.

Design Flexible Experiments that Scale

SCALE FROM PILOT PROJECTS TO MILLIONS OF CELLS

Many studies require collection of samples over time, and increasingly require larger sample and cell numbers. Other single-cell technologies require samples to be analyzed immediately and are limited by the number of samples that can be analyzed together, which can introduce batch effects. Regardless of scale, Evercode technology has a kit to fit every study.

WT Mini v2

10K CELLS | 12 SAMPLES

Generate proof of concept results for larger studies, grants or just to try the technology risk-free

WT v2

100K CELLS | 48 SAMPLES

Comprehensive gene profiling across samples, replicates, or timepoints for most studies

WT Mega v2

1M CELLS | 96 SAMPLES

Expand your science or capabilities by profiling up to 1 million cells per experiment

A SIMPLER WORKFLOW WITH FIXATION

Fix cells or nuclei to lock in the biology until your experiment is ready. Fixation prevents RNA degradation, enabling flexible sample collection and processing. Samples retained 97% of gene and 96% transcript detection 6 months post-fixation.

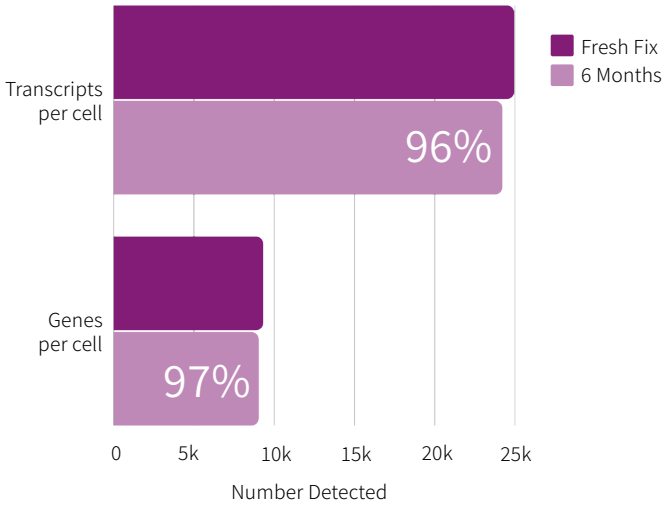


Figure 3: Evercode Fixation Stabilizes Transcriptome Profiles. Samples were fixed with Evercode Cell Fixation v1 and processed with Evercode WT v1 immediately or after six months of storage at -80°C. Gene and transcript detection was not significantly impacted.

LOW MULTIPLET RATES AT EVERY SCALE

Multiplets are a nuisance of single cell RNA-Seq approaches that complicate data analysis. Because Evercode relies on each cell/nuclei as a reaction vessel rather than droplets, it has a dramatically lower multiplet rate across a wide range of cell/nuclei inputs.

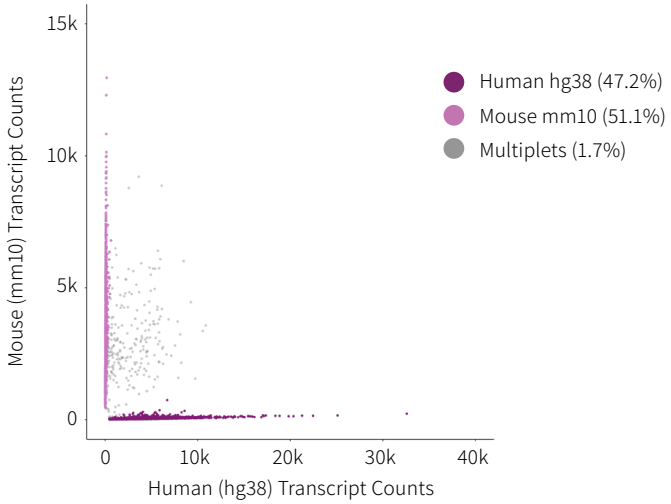


Figure 4: Evercode Produces Low Multiplet Rates. A human-mouse species mixing experiment using Evercode WT v2 produced an observed multiplet rate of only 1.7% per 100,000 cells — substantially lower than droplet-based technologies.

COMPREHENSIVE PROFILING OF UP TO 1 MILLION CELLS IN A SINGLE EXPERIMENT

To demonstrate the flexibility and scalability of the Evercode Whole Transcriptome products, twenty-four PBMC samples from donors with Type 1 Diabetes (T1D) and controls were analyzed. Over a 3 week period, PBMC samples were collected, fixed, and stored at -80°C. As the samples could be easily fixed over the collection period, samples were then all processed together in a single Evercode WT Mega kit. Over 27,000 cells were captured from each sample for almost 1 million cells total. Low frequency cell types that are more difficult to capture in smaller scRNA-seq assays were identified, including subtypes of classical dendritic cells (cDC1, cDC2) and plasmacytoid dendritic cells (pDC). Further analysis identified significant heterogeneity in cell type proportions between and within T1D status. This highlights the importance of biological replicates in scRNA-seq studies of heterogeneous diseases like T1D.

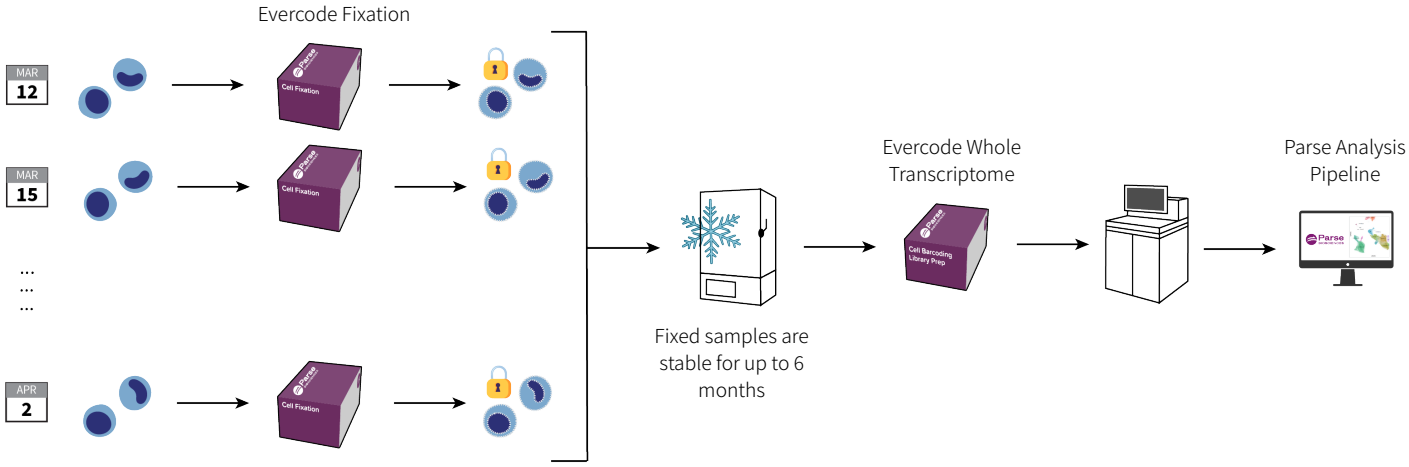


Figure 5. Study Design. Samples were collected and fixed at the time of collection over a 3 week period and stored at -80°C. Once all 24 samples were collected, they were processed with a single Evercode WT Mega v1 kit to generate approximately 1 million individually barcoded cells. All 16 sublibraries were sequenced together on an S4 flow cell with an Illumina Novaseq 6000.

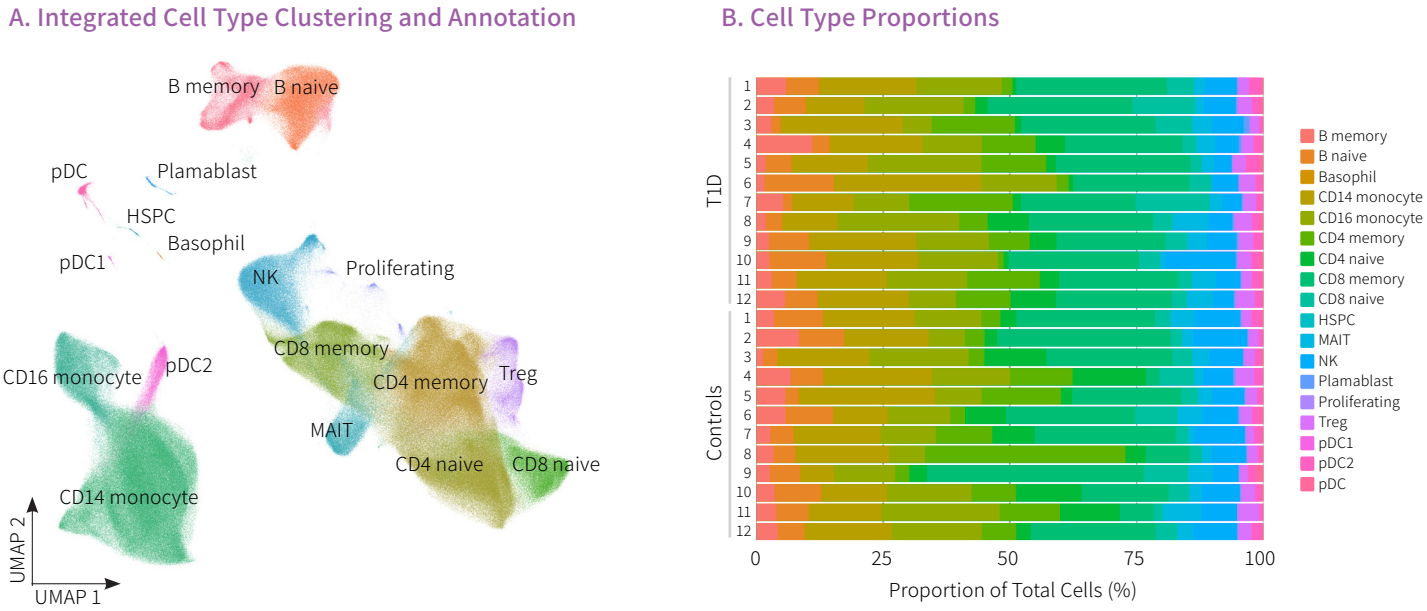


Figure 6: One million PBMCs from T1D and Control Samples. (A) Sequencing data were processed with the Parse Biosciences pipeline, integrated, co-clustered, annotated, and visualized as a UMAP. (B) Bar chart with the proportion of each cell type within each sample. Several low frequency cell types (cDC1, cDC2, pDC) can be distinguished both in the clustering and bar chart.

Resolve More Biology from Every Cell

COMPARISON OF EVERCODE WT V2 TO DROPLET-BASED TECHNOLOGY

Parse Biosciences Evercode WT v2 combinatorial barcoding technology was compared to the droplet-based 10x Genomics™ Chromium™ Next GEM Single Cell 3' Kit v3.1 with mouse brain nuclei. This heterogeneous sample type has been the focus of an array of cell atlas projects and individual researchers.

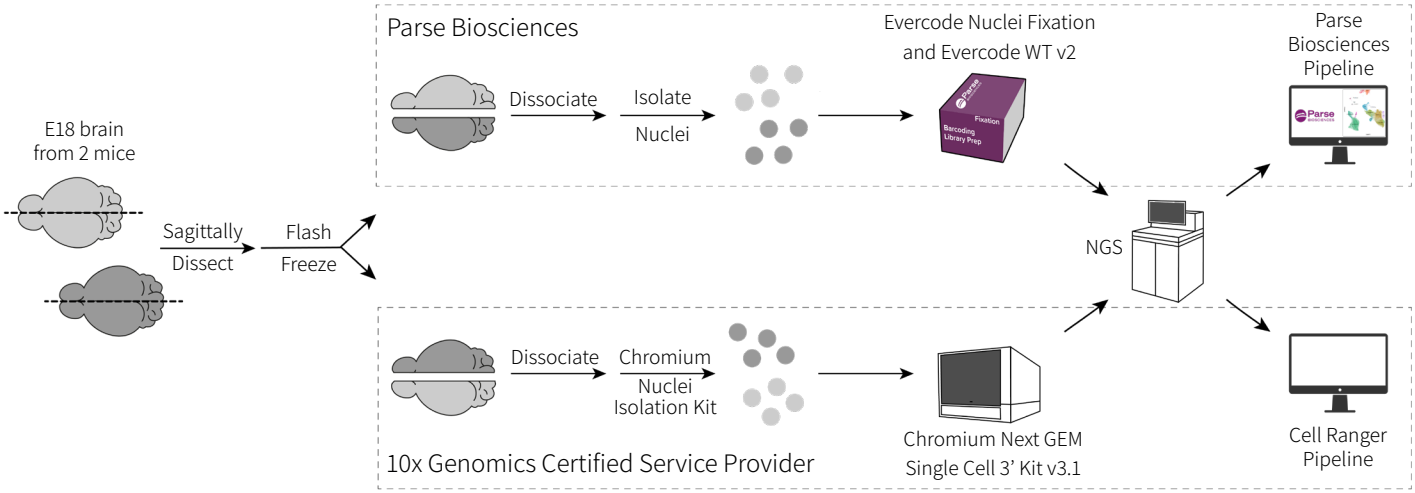


Figure 7. Comparison Study Design. Two E18 embryonic C57/BI6 samples were collected, sagittally dissected into 2 halves, and flash frozen by a third-party tissue vendor. Half of each brain was shipped to a 10x Genomics Certified Service Provider, and they isolated nuclei with the Chromium Nuclei Isolation Kit and created sequencing libraries with the Chromium Next GEM Single Cell 3' GEM Library & Gel Bead Kit v3.1. The other halves of each brain were processed by Parse Biosciences for nuclei isolation with a dounce homogenizer, fixation with Evercode Nuclei Fixation v2, and library preparation with Evercode WT v2. Sequencing libraries from each technology were sequenced by a third-party. The sequencing data were analyzed with each manufacturer's data analysis pipeline.

REPRODUCIBLY HIGHER SENSITIVITY WITH EVERCODE WT V2

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 the common read depth target of 20,000 reads/cell. Increased sensitivity enables better detection of lowly expressed genes, resulting in more comprehensive annotation of cell types.

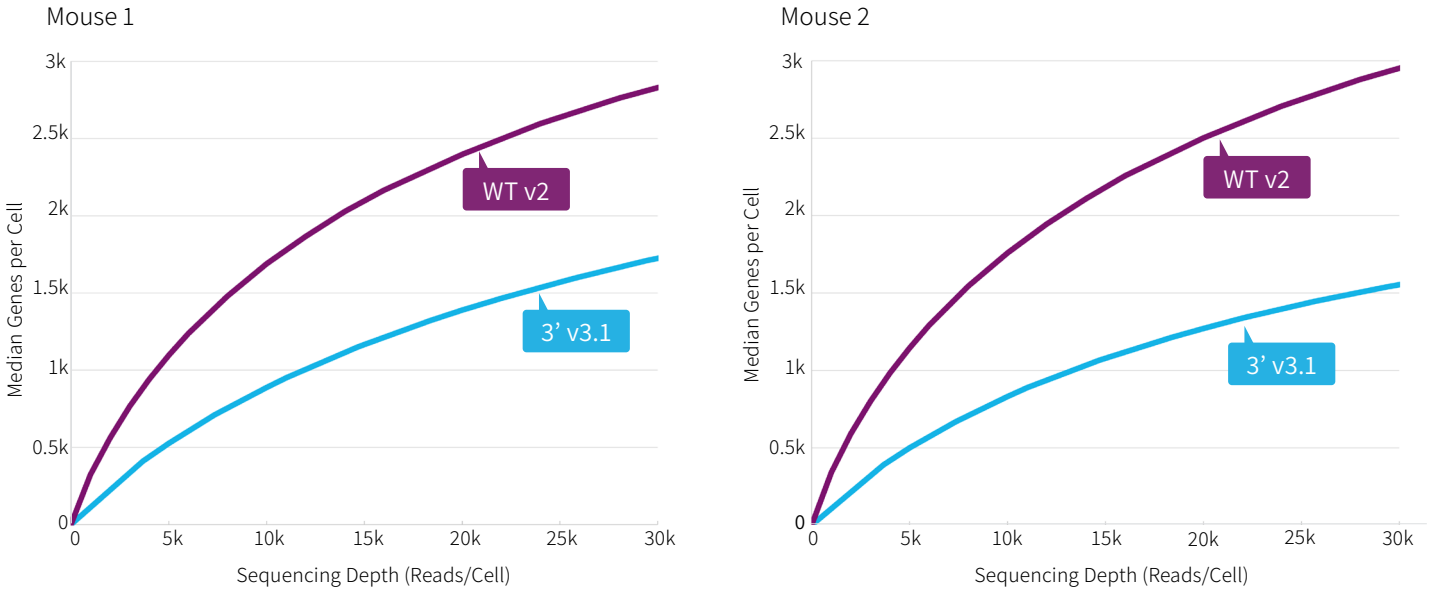
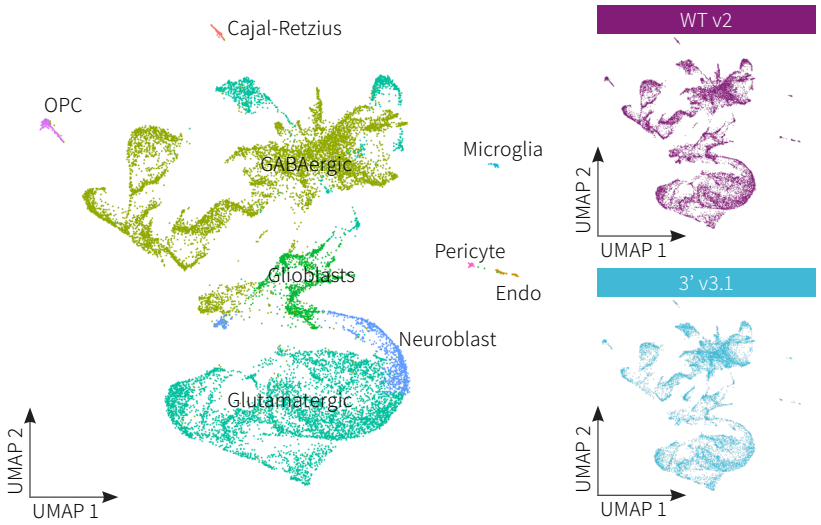


Figure 8. Gene Detection Sensitivity. Median genes detected per nuclei across different sequencing depths for mouse brain 1 (left) and mouse brain 2 (right). Each mouse brain was sagitally dissected into two halves and sent to different laboratories for processing with either Evercode WT v2 or 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 technologies and analyzed by their respective data analysis pipelines.

RECOVER THE SAME MAJOR CELL TYPE PROPORTIONS AS DROPLET TECHNOLOGY

A. Integrated Evercode WT v2 and Chromium Next GEM 3' v3.1 Clustering



B. Relative abundance of cell types

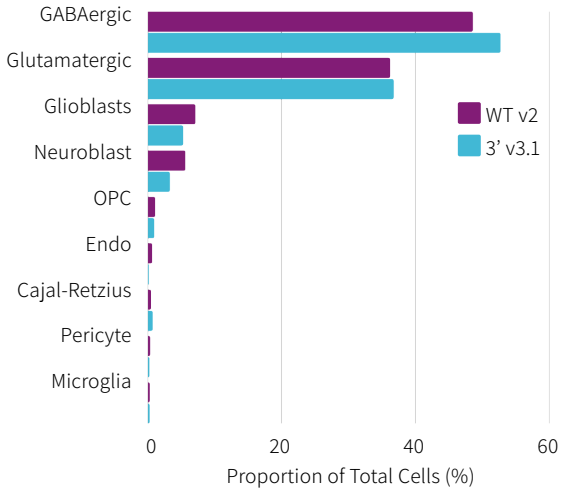
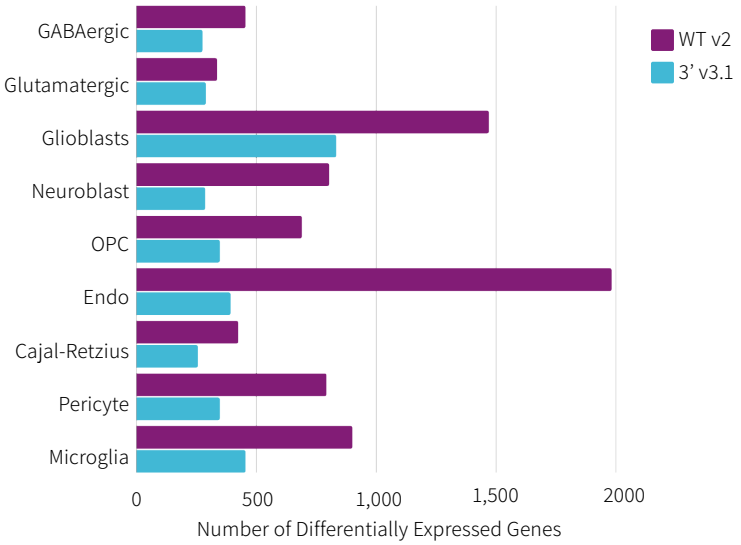


Figure 9. Gene Expression Profile Comparison. (A) After downsampling to the same sequencing depth, data from 17,339 nuclei from Evercode WT v2 and 12,967 nuclei from Chromium Next GEM Single Cell 3' Kit v3.1 were integrated, clustered, and visualized with annotations. (B) A comparison of relative abundance of the major cell types (presented as % of total cells) was performed to confirm concordance of expression between the technologies.

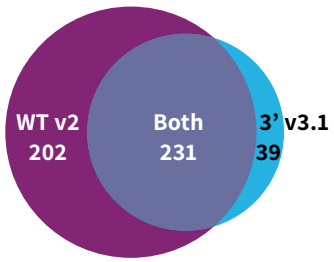
EVERCODE WT V2 DELIVERS HIGHER RESOLUTION DATA

Evercode WT v2 consistently detected more differentially expressed (DE) genes than Chromium Next GEM 3' v3.1 across all cell types. Most differentially DE detected by Chromium Next GEM 3' v3.1 were also detected with Evercode WT v2. When cell types are defined by more DE genes, researchers can gain a deeper understanding of the biology of their samples.

A. Differentially expressed genes that define cell types



B. GABAergic Neurons



C. Microglia

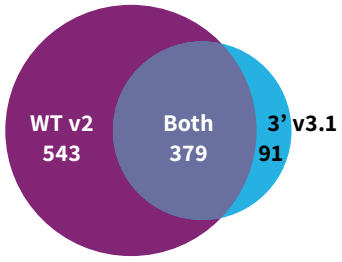


Figure 10. Cell Type Defining Differential Gene Expression. (A) The number of differentially expressed genes for each major cell type, ordered by abundance of the cell type. The uniqueness of differentially expressed genes in the highest (B, GABAergic Neurons) and lowest (C, Microglia) abundant cell 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.

PRODUCT ORDERING INFORMATION

PRODUCT	PART NUMBER
Evercode WT Mini v2 Up to 10,000 cells or nuclei and up to 12 samples	ECW02010
Evercode WT v2 Up to 100,000 cells or nuclei and up to 48 samples	ECW02030
Evercode WT Mega v2 Up to 1,000,000 cells or nuclei and up to 96 samples	ECW02050
Evercode Cell Fixation v2 Up to 4 samples	ECF2001
Evercode Nuclei Fixation v2 Up to 4 samples	ECF2003

We Love Hearing from You

Website: parsebiosciences.com

Email: info@parsebiosciences.com

Scan this code to learn more and find the data presented here.