Evercode™ Whole Transcriptome

Scalable Single Cell Sequencing, No Instrument Required

Easy to Get Started
Flexible Workflow
Unmatched Sensitivity

PARSE BIOSCIENCES

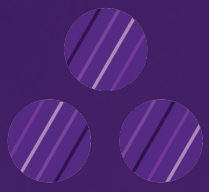
Single Cell RNA-Seq in Any Lab

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

Step 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).

parsebiosciences.com | info@parsebiosciences.com

Exponentially Scalable

Evercode's combinatorial barcoding enables you to dramatically scale up the cells and samples per experiment.

Unmatched Data Quality

Better detect lowly expressed genes and avoid ambient RNA common in droplet-based single cell sequencing.

No Instrument Required

If you have a centrifuge, thermal cycler, and some pipettes, you're ready to go.

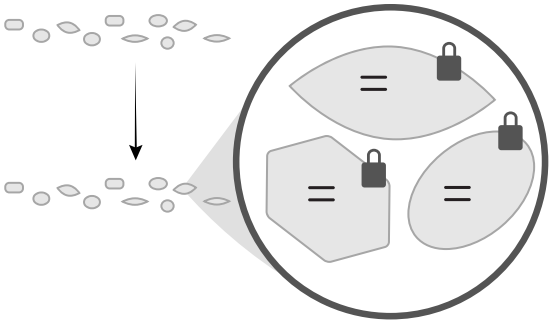
Cell and Nuclei Fixation

Fix and store samples as they come in for up to 6 months and then run together later on your schedule. Ideal for time-courses and cross-site collaborations.

PARSE BIOSCIENCES

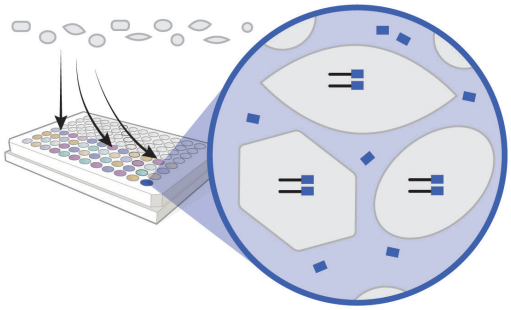
Fixation

Cells/nuclei samples are fixed and permeabilized.



Barcoding Round 1

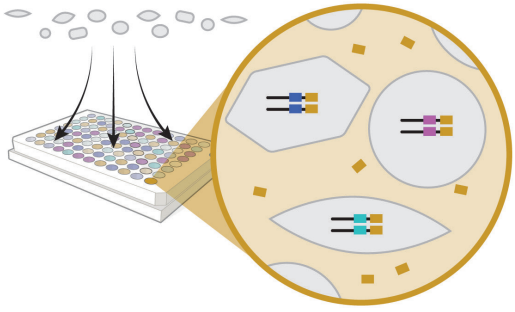
Split | Fixed cells/nuclei are distributed into wells, and the first sample-specific barcodes are added by in-cell reverse transcription.



Pool | All the cells are pooled together.

Barcoding Round 2

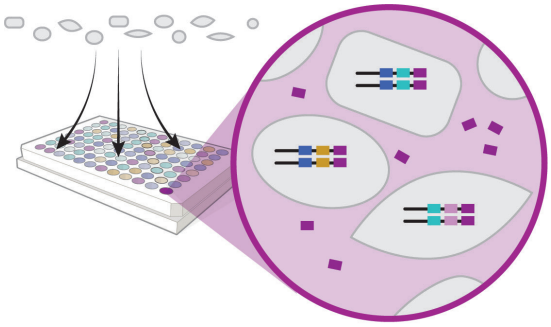
Split | The pooled cells are distributed across a plate, and an in-cell ligation adds the second barcode.



Pool | All the cells are pooled together.

Barcoding Round 3

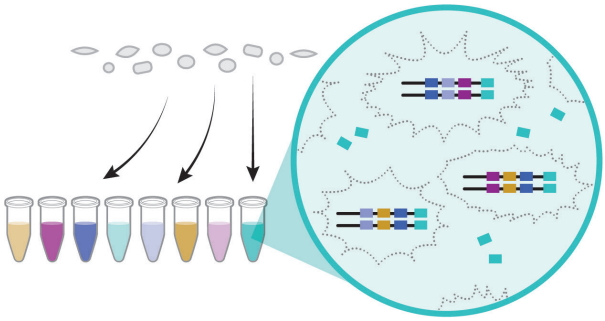
Split | The pooled cells are again distributed across a plate, and a third barcode is added via in-cell ligation reaction.



Pool | All the cells are pooled together.

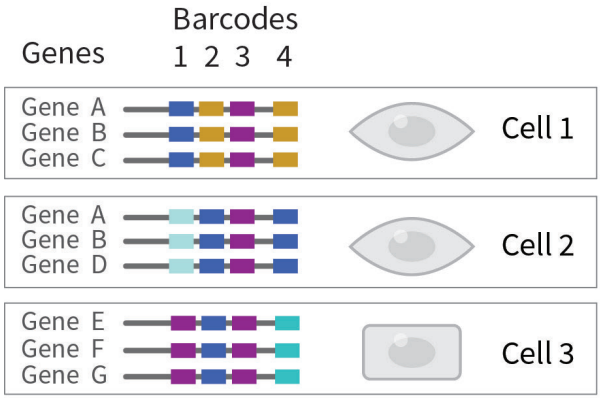
Lysis and Library Prep

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



Sequencing and Analysis

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



No Instrument Required with Combinatorial Barcoding

The Cell Is the Reaction Comparment

Cells are first fixed and permeabilized, turning them into their own reaction vessels, removing the need to capture individual cells in droplets or microwells. The split-pool barcoding process then labels cells with an exponentially large number of barcode combinations making it possible to easily scale beyond other technologies.

Single Cell Data Analysis Made Easy with Trailmaker

Every kit comes with our data analysis platform, Trailmaker™, which transforms sequencing output (FASTQ) into understandable results and publication-ready figures. Trailmaker enables users to process, integrate, explore, plot, and share Evercode Whole Transcriptome data anytime and anywhere with push-button analytics.

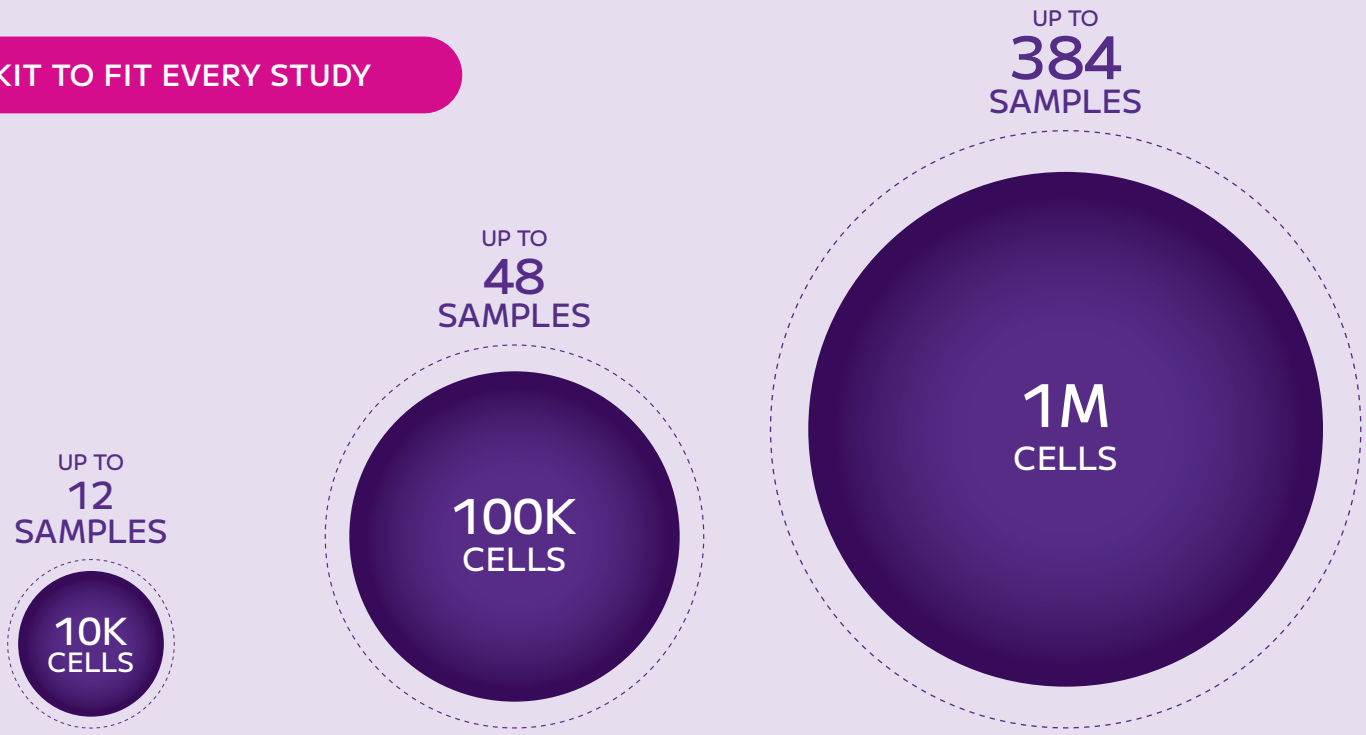
PARSE BIOSCIENCES

Seamlessly Scale Your Single Cell Projects

From Pilot to Millions of Cells & Hundreds of Samples

Regardless of scale, Evercode technology has a kit to fit every study. Whether you want to generate proof of concept results or perform cell atlassing studies, our technology labels cells with thousands to millions of barcode combinations making it possible to easily scale beyond other technologies.

A KIT TO FIT EVERY STUDY



Evercode WT Mini

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

Evercode WT

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

Evercode WT Mega

Expand your science by profiling up to 1 million cells per run across 100s of conditions or samples

parsebiosciences.com | **info@parsebiosciences.com**

Fix Now. Run Later.

Many studies require collection of samples over time, and increasingly require larger sample and cell numbers. Simplify your process by fixing samples immediately upon collection and storing them for up to 6 months until they can be processed all together.

Fixation of Nuclei and Cells

Easily work with collaborators and service providers

Minimize bias from batch effects

Conduct time-course studies conveniently

PARSE BIOSCIENCES

Maximize Research Impact Through Efficient Workflow

- Capture the full heteregenity of complex samples
- Increase statistical power by including more replicates

384 samples fixed » **1** day

1 million cells » **2** days

Effortlessly Scale to Millions of Cells

1,084,202 PBMCS IN 1 EXPERIMENT

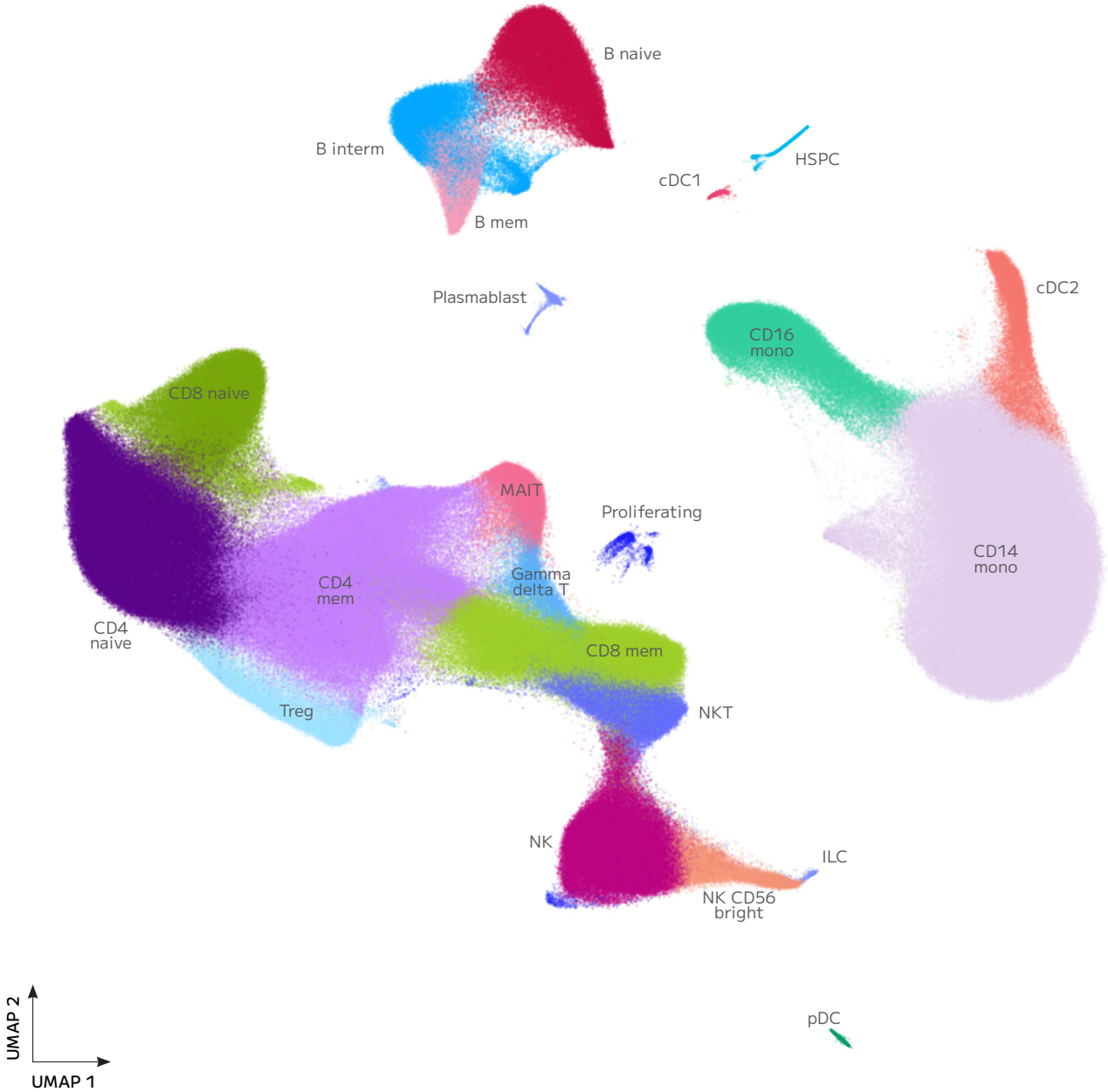


Figure 1. One Million PBMCs. PBMCs isolated from 12 donors were fixed across 48 wells with the Evercode Cell Fixation v3 high throughput plate-based workflow. Fixed samples were processed with a single Evercode WT Mega v3 kit to generate 1,084,202 barcoded cells. Libraries were sequenced a 25B flow cell with an Illumina Novaseq X. Sequencing data were processed with the Parse Biosciences pipeline, integrated, co-clustered, annotated, and visualized as a UMAP.

PARSE BIOSCIENCES

The Most Scalable Technology

More Cells, More Samples, One Kit

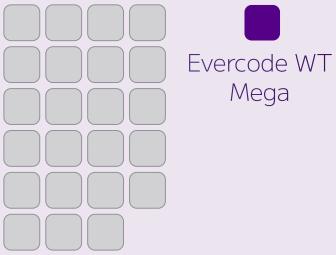
Parse Biosciences Evercode combinatorial barcoding technology scales exponentially without increasing effort or risking batch effects. Droplet-based assays are limited by scaling linearly.

Pilot Project
40,000 cells

Evercode WT

4 Lanes
Chromium Next GEM
Single Cell 3' Kit

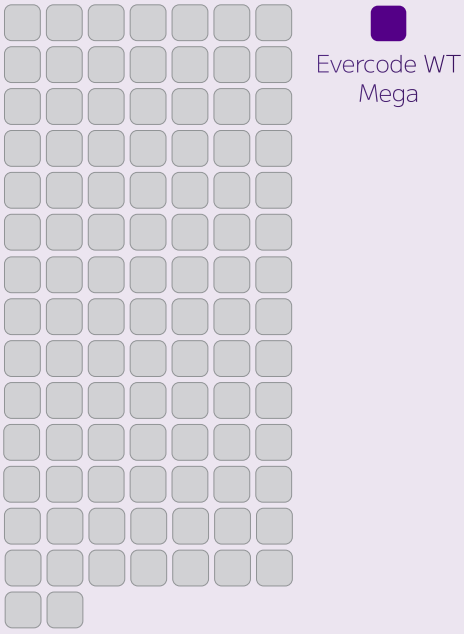
Average Published Study in 2022
230,000 cells



Evercode WT
Mega

23 Lanes
Chromium Next GEM
Single Cell 3' Kit

Cell Atlas
1,000,000 cells



Evercode WT
Mega

100 Lanes
Chromium Next GEM
Single Cell 3' Kit

More Genes, Less Sequencing

Evercode Whole Transcriptome v3 technology shows higher gene detection compared to the droplet-based 10x Genomics™ Chromium™ GEM-X Single Cell 3′ Kit v4 across sample types.

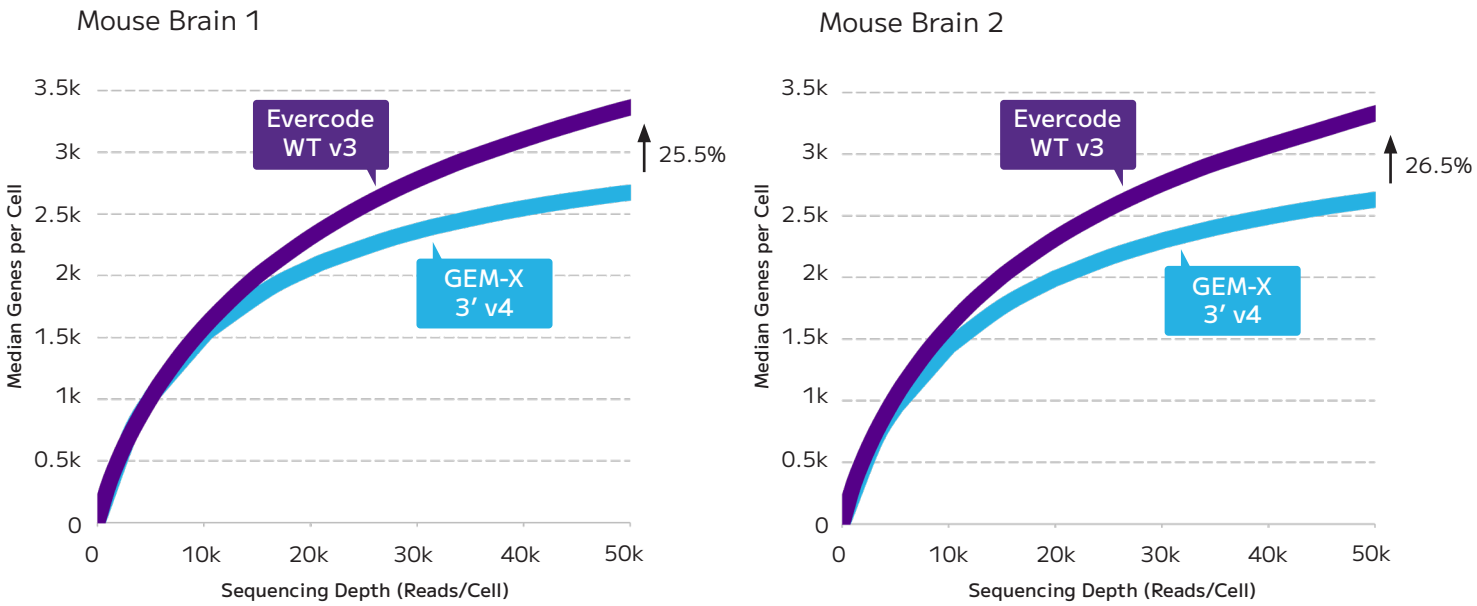


Figure 2. Gene Detection. Median genes detected per nuclei across different sequencing depths for mouse brain 1 (left) and mouse brain 2 (right). Each mouse brain was sagitally dissected into two halves and sent to different laboratories for processing with either Evercode WT v3 or Chromium GEM-X 3′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

PARSE BIOSCIENCES

Transition Seamlessly to Evercode

Integrates with Existing Data

Integration of the data from Evercode WT v3 and GEM-X 3′ v4 resulted in highly concordant clustering and cell proportions, indicating both technologies result in unbiased capture of cell types.

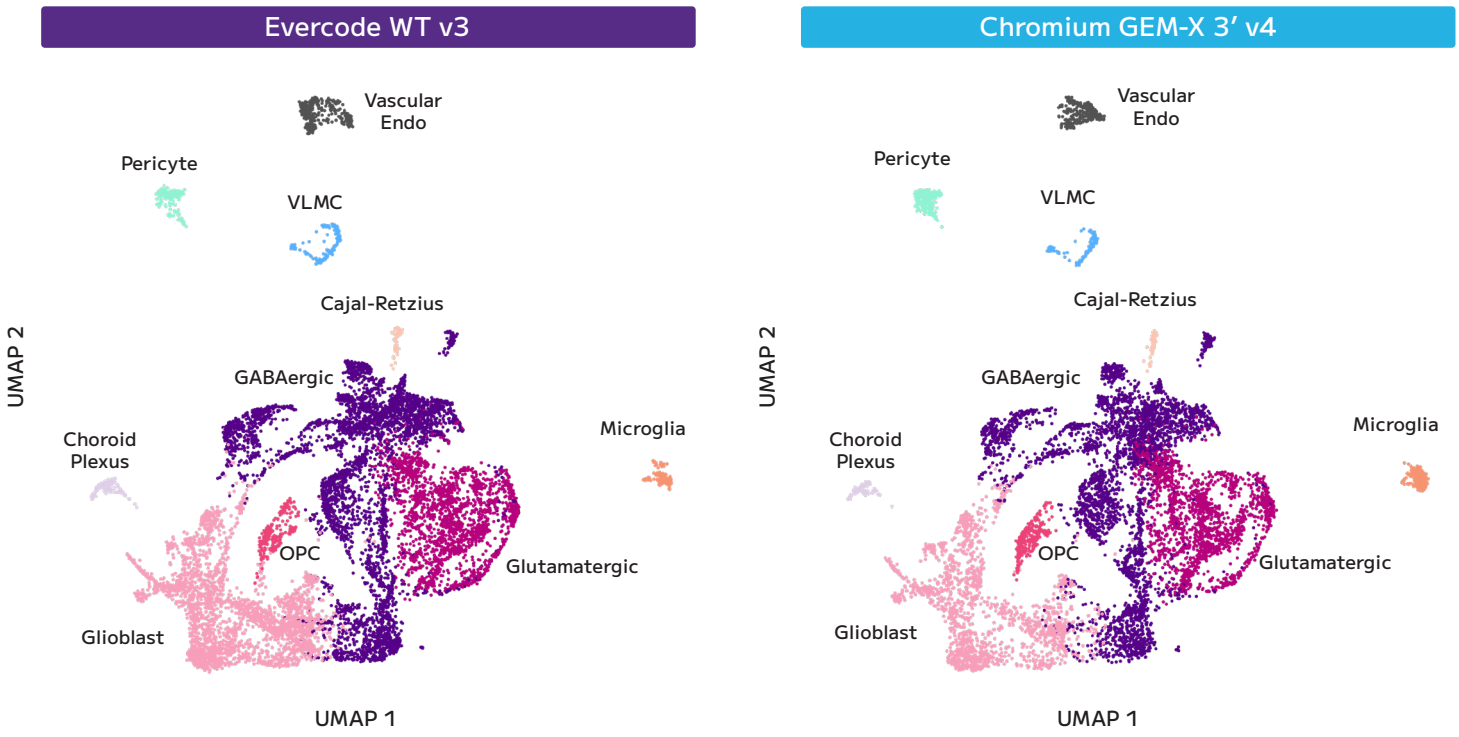
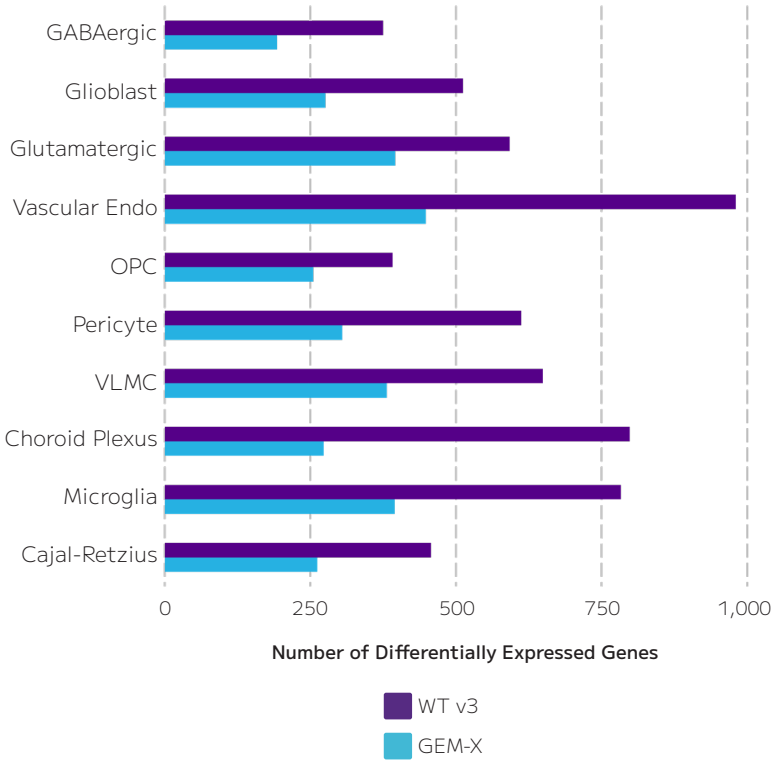


Figure 3. Integrated Evercode WT v3 and Chromium GEM-X 3′ v4 Clustering. 10,000 nuclei from Evercode WT v3 and 10,000 nuclei from Chromium GEM-X 3′ v4 were integrated, clustered, annotated with Trailmaker, and visualized separately in annotated UMAPs.

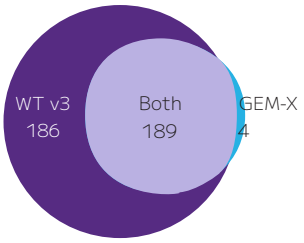
Higher Resolution, More Biology

Evercode WT v3 consistently detected more differentially expressed genes (DEG) than GEM-X 3′ v4. Most DEG detected in GEM-X 3′ v4 data were also detected with Evercode WT.

A. Differentially expressed genes that define cell types



B. GABAergic Neurons



C. Cajal-Retzius

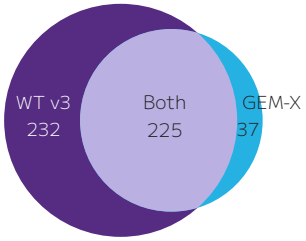


Figure 4. Comparison of Differentially Expressed Genes. (A) The number of differentially expressed genes for each major cell type, ordered by abundance of the cell type. The uniqueness of differentially expressed genes in the highest (B, GABAergic Neurons) and lowest (C, Cajal-Retzius) abundant cell 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.

Analyze Cleaner Data

Wash Away Ambient RNA

The cell is the reaction compartment with Evercode technology, which means there's no droplets to encapsulate ambient RNA. Prior to library prep, any free floating RNA is washed away leaving behind just the RNA molecules within cells. The pervasive detection of hemoglobin transcripts in the Next GEM 3′ v3.1 data suggests that lysed RBCs are a major source of ambient RNA contamination, which is not observed at all with Evercode.

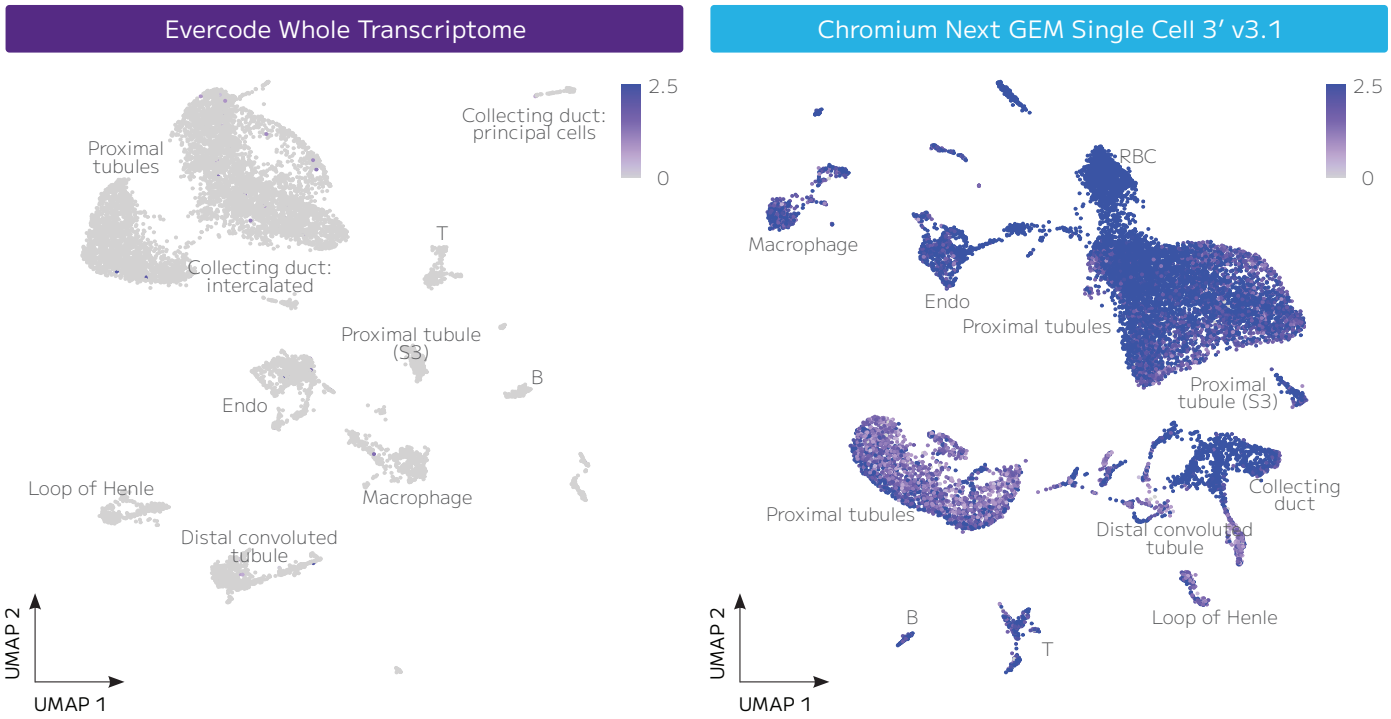


Figure 5. Clustering and Hemoglobin Expression Comparison. Mouse kidney tissue was dissociated into a single cell solution with a Singulator 100 (S2 Genomics). The samples were strained and red blood cells (RBCs) were lysed. The sample was split and half of the sample was prepared with the 10x Genomics Next GEM Single Cell 3′ Kit v3.1. The remaining half was fixed with Evercode Cell Fixation v2 and shipped for further processing with Evercode WT v2. Sequencing data were analyzed with each manufacturer's respective analysis pipeline. 9,256 mouse kidney cells from each technology were independently clustered with Seurat and visualized as UMAPs. Expression of hemoglobin alpha, adult chain 1 (Hba-a1) is shown for both technologies.

Trusted by 2,400+ Labs and Growing

Advancing Research Globally

The breakneck rate of adoption by single cell researchers is a testament to the utility of Evercode Whole Transcriptome data.

For your next experiment, ask us how we can help you get more out of your experiments - smashing through barriers and re-defining what's possible.

PARSE BIOSCIENCES

PRODUCT	PART NUMBER
Evercode WT Mini v3 Up to 10,000 cells or nuclei and up to 12 samples	ECWT3100
Evercode WT v3 Up to 100,000 cells or nuclei and up to 48 samples	ECWT3300
Evercode WT Mega v3 Up to 1,000,000 cells or nuclei	
Up to 96 samples	ECWT3500
Up to 384 samples	ECWT3530
Evercode Cell or Nuclei Fixation v3 Up to 1,000,000 cells or nuceli	
Up to 12 samples	ECFC3300
High throughput fixation for 96 samples	ECFC3501
Evercode Cell or Nuclei Low Input Fixation v3 Input as low as 10,000 cells or nuclei	
Up to 12 samples	ECLC3301
High throughput fixation for 96 samples	ECLC3501

More Cells, More Samples, More Clarity

parsebiosciences.com | **info@parsebiosciences.com**

Explore more resources