Technical Note
Evercode Whole Transcriptome

Comparison of Evercode™ WT v4 and Chromium™ GEM-X Single Cell 3′ Kit v4 in Human PBMCs

Introduction

Here we present the results of a comparative evaluation of single cell RNA-seq technologies using human peripheral blood mononuclear cells (PBMCs). The study compares droplet-based microfluidics (10x Genomics Chromium™ GEM-X Single Cell 3′ Kit v4) and combinatorial barcoding (Parse Biosciences Evercode™ WT v4) workflows. Aliquots derived from the same PBMC donors were processed independently with each technology to enable a controlled assessment of overall performance.

Comparison Highlights

- Sensitivity comparison shows a marked increase in transcript detection in Evercode WT.
- Cell type proportions are equivalently represented.
- Reduced ambient RNA is observed with Evercode WT.
- Evercode exhibits significant decrease in mitochondrial and ribosomal reads.

EXPERIMENTAL DESIGN

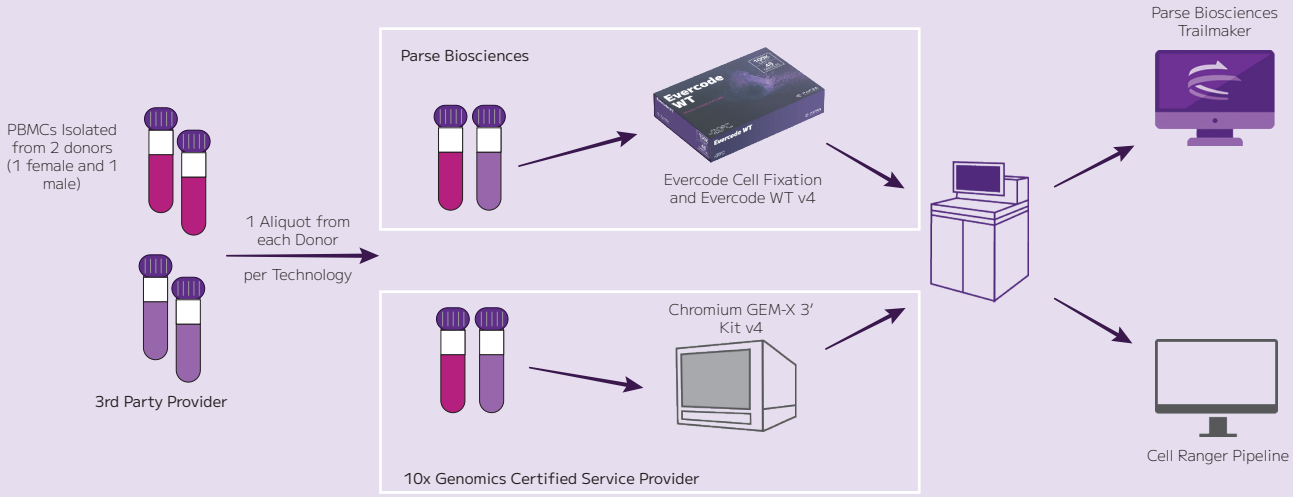

Figure 1. Experimental Design. Frozen PBMCs from two donors (one female and one male) were obtained from a third-party provider. Aliquots derived from each donor were distributed to separate laboratories for processing. One aliquot per donor was shipped to a 10x Genomics Certified Service Provider for cell preparation and library construction using the Chromium GEM-X Single Cell 3′ Kit v4. Parallel aliquots were processed by Parse Biosciences using the Evercode WT v4 workflow. Sequencing libraries from both technologies were sequenced on the same sequencer model, and data were analyzed using each manufacturer's respective analysis pipeline.

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Methods

Sample Collection

Peripheral blood mononuclear cells (PBMCs) were sourced as frozen cryovials from a third-party provider. Aliquots from each of two PBMC donors (one female and one male) were distributed to the respective laboratories for downstream processing and library preparation. Aliquots originating from the same donor cryovial lot were used across workflows to maintain comparable starting material between technologies.

10x Genomics Chromium GEM-X 3' v4

A vial of PBMCs from each donor was shipped to a 10x Genomics Certified Service Provider for sample and library preparation. With a target of 40,000 cells per donor, a Chromium GEM-X Single Cell 3' GEM Library & Gel Bead Kit v4 was used to partition and prepare sequencing libraries. All sample preparation and library construction steps were performed according to the manufacturer's recommended protocols by the Certified Service Provider.

Parse Biosciences Evercode WT v4

PBMC samples were fixed using the Parse Biosciences high-throughput fixation kit. Fixed cells were aliquoted and stored at -80°C prior to library preparation. Whole transcriptome sequencing libraries were prepared using the Evercode WT v4 workflow according to the user guide. Whole transcriptome sequencing libraries were prepared with a target of 50,000 cells per donor using Evercode WT v4.

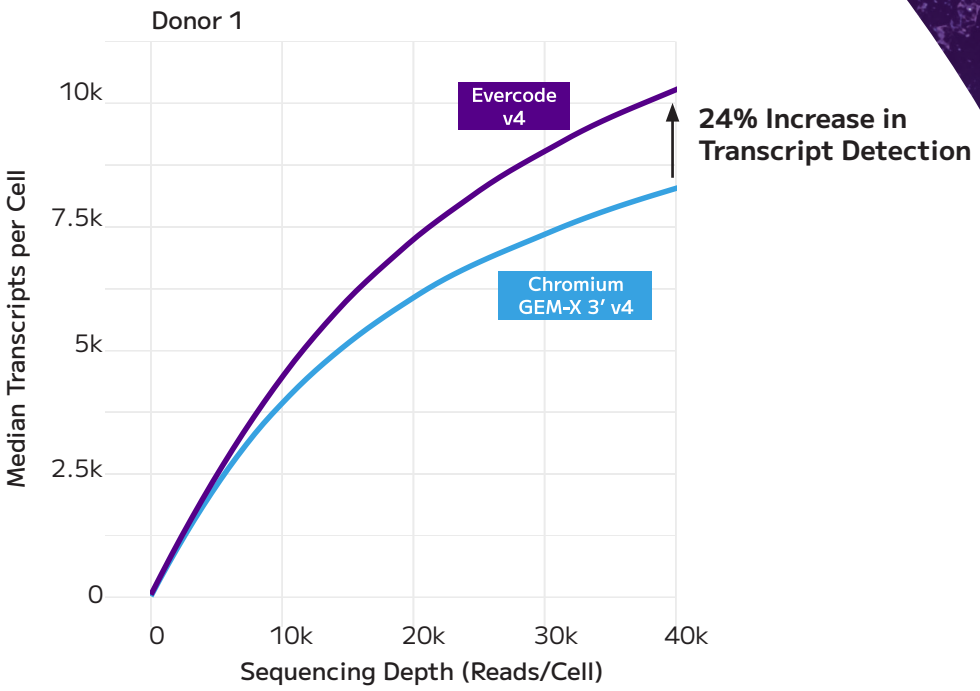
Sequencing and Data Analysis

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 analyzed with Cell Ranger™ v10.0.0, and the Parse Biosciences data were analyzed with the Parse Biosciences analysis pipeline v1.7.0.

Integration and Cell Type Annotation

All libraries were downsampled to 40,000 mean reads per cell. The Parse Evercode WT v4 and 10x Genomics GEM-X 3' v4 datasets were integrated using Harmony. Following integration, the data were visualized using UMAP. Clusters were identified using the Louvain algorithm, and marker genes were determined for each cluster. Cell type identities were assigned based on established PBMC marker genes. 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 log2 fold change and adjusted $p<0.001$.

MORE GENES, LESS SEQUENCING



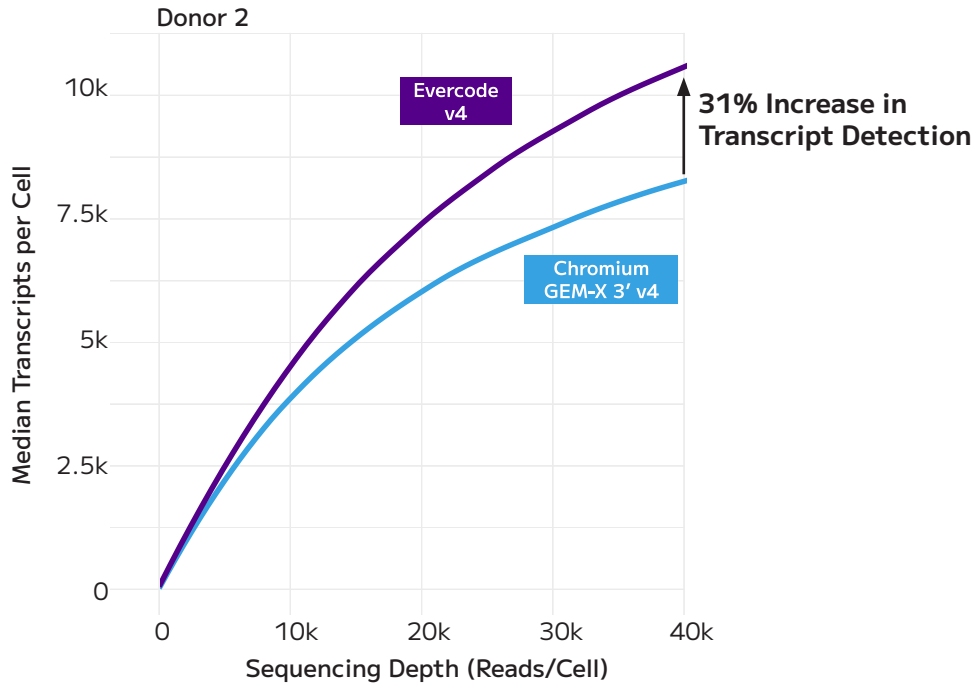


Figure 2. Gene Detection. Median genes detected per cell across different sequencing depths for PBMC donor 1 (top) and PBMC donor 2 (bottom). Aliquots derived from the same donor cryovial lot were distributed to separate laboratories for processing with either Evercode WT v4 or Chromium GEM-X 3' v4 workflows and analyzed using their respective data analysis pipelines.

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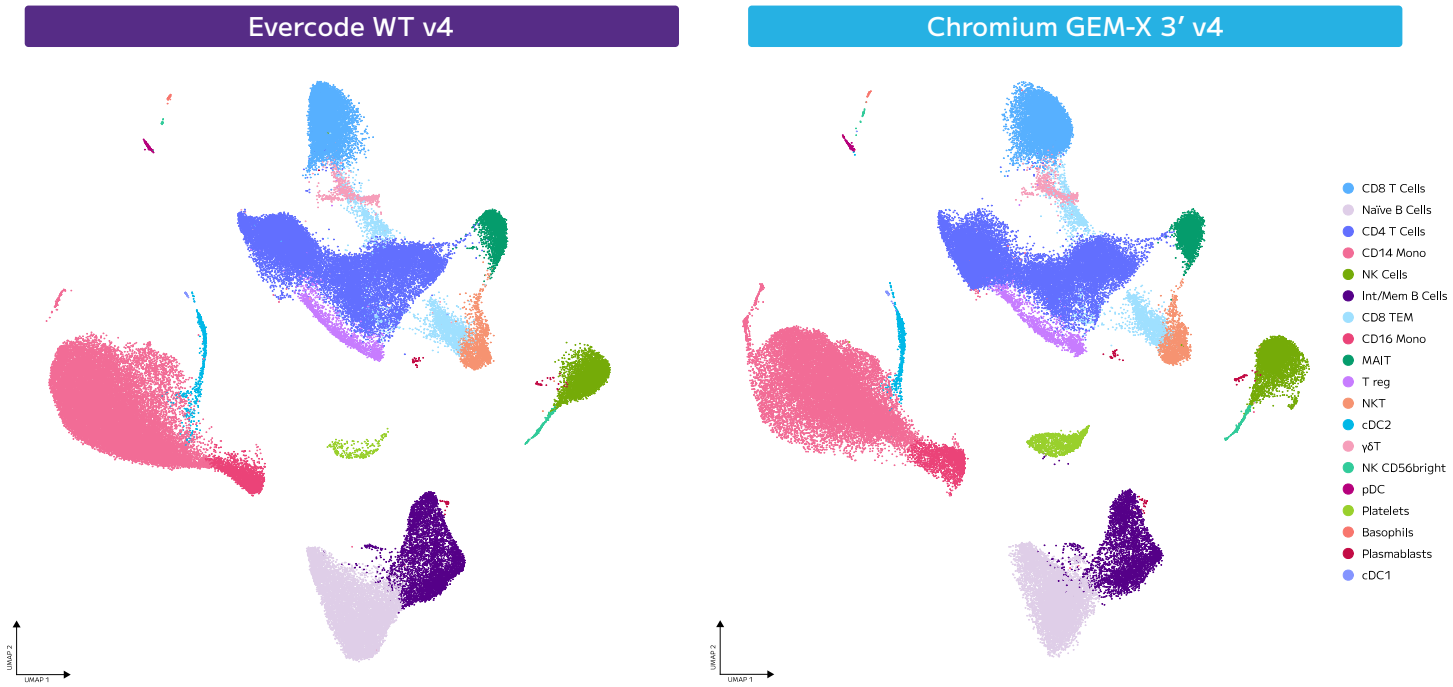
Consistent Gene Expression and Proportions of Cell Types

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



B. Comparison of relative abundance of cell types

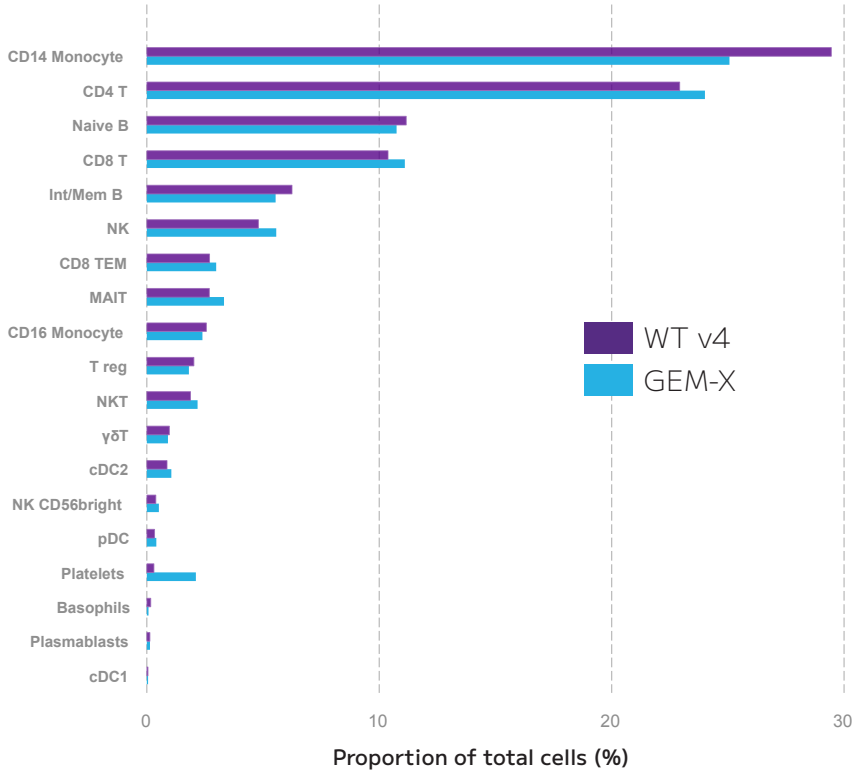


Figure 3. Gene Expression Profile Comparison.
(A) 40,000 cells from Evercode WT v4 and 40,000 cells from Chromium GEM-X 3' v4 were integrated, clustered, annotated, and visualized separately in annotated UMAPs. (B) A comparison of relative abundance of the major cell types (presented as % of total cells) was performed to confirm concordance of expression between the technologies.

Analyze Cleaner Data

Global stress-associated gene expression

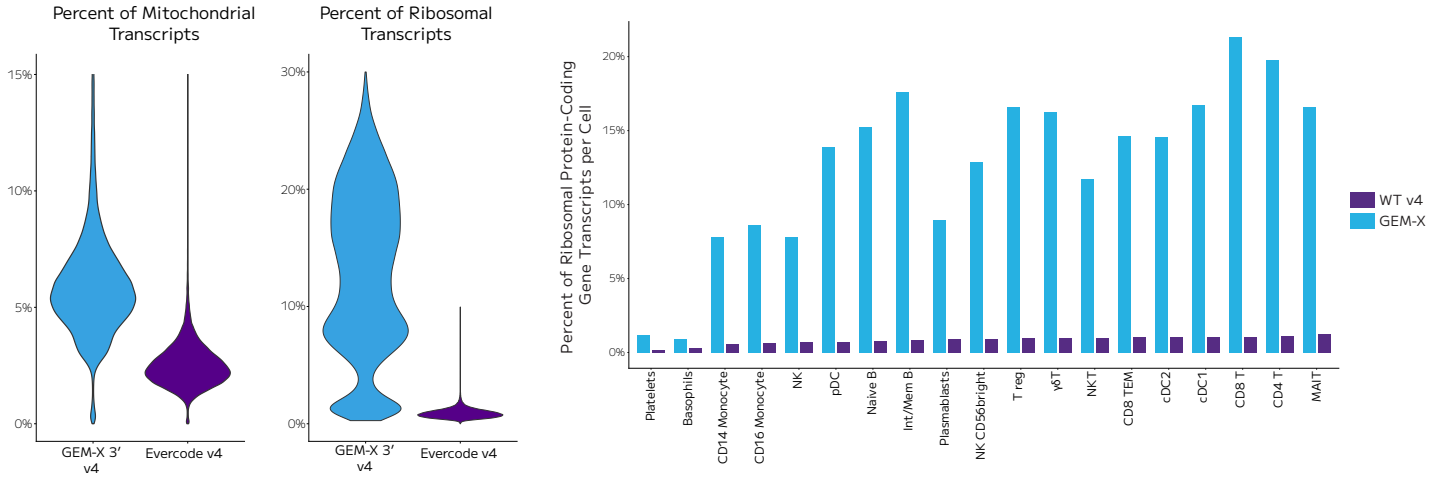


Figure 4. Increased Mitochondrial and Ribosomal Signal in Chromium GEM-X 3' v4. Initial standard QC filtering has been applied for both technologies, including removing multiplets and filtering out cells with 10% or more mitochondrial transcripts to show what would still remain in both technologies. (A) Violin plots showing the distribution of mitochondrial and ribosomal reads across cells processed with Chromium GEM-X 3' v4 and Evercode WT v4. (B) Percentage of ribosomal protein gene transcripts across cell-type clusters, showing consistently elevated ribosomal signal in Chromium GEM-X 3' v4 across clusters, potentially reflecting increased cellular stress associated with sample processing constraints.

Comparison of ambient RNA across technologies

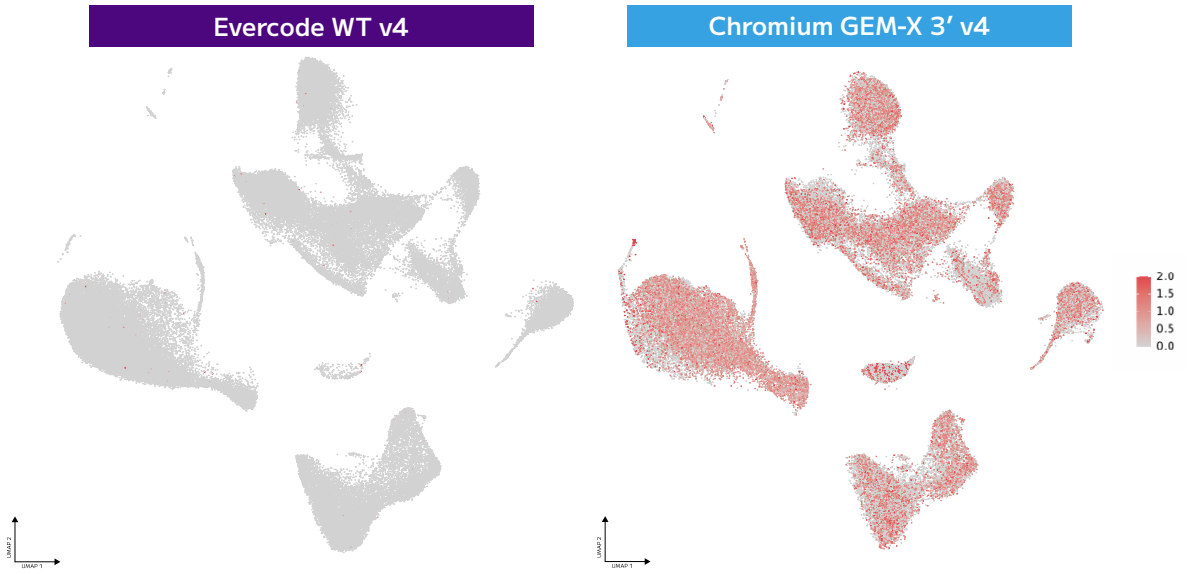


Figure 5. Reduced Ambient RNA Expression in Evercode. PBMCs processed with Evercode WT v4 and Chromium GEM-X 3' v4 were independently clustered, annotated, and visualized using UMAP. Expression of hemoglobin beta (HBB) is shown for both technologies as a qualitative assessment of ambient RNA.

Results

Sensitivity
Across sequencing depths, Evercode WT v4 detected more transcripts per cell than Chromium GEM-X 3′ v4 (Figure 2). At 40,000 reads per cell, Evercode WT v4 detected an average of 27.5% more transcripts per cell compared to Chromium GEM-X 3′ v4 across both PBMC donors. Increased transcript detection was consistent across sequencing depths and maintained following downsampling to matched read depths, indicating improved sensitivity per cell.

Cell Proportions
Integration of Evercode WT v4 and Chromium GEM-X 3′ v4 datasets resulted in concordant clustering and similar proportions of major PBMC populations (Figure 3). These results indicate that both workflows capture comparable cellular composition from matched donor material.

Cleaner Data
Expression of hemoglobin beta (HBB) was evaluated as a marker of ambient RNA signal (Figure 4). Evercode WT v4 exhibited reduced background HBB expression relative to Chromium GEM-X 3′ v4, consistent with lower ambient RNA contamination. HBB serves as an easy to detect marker for the larger underlying issue of pervasive ambient RNA contamination of transcripts from any gene, which are much harder to detect and filter out. Ambient RNA signal can influence clustering, differential gene expression analysis, gene marker detection, and overall data quality.

Chromium GEM-X 3′ v4 exhibited elevated mitochondrial and ribosomal read counts relative to Evercode WT v4 across all PBMC clusters (Figure 5). Evercode WT v4 maintained lower stress-associated expression patterns.

Conclusion

This evaluation compared two reverse transcription–based single cell whole transcriptome technologies using matched PBMC donor material.

Evercode WT v4 demonstrated increased transcript detection sensitivity at 40,000 reads per cell while maintaining comparable cell type composition. Evercode WT v4 also exhibited reduced ambient hemoglobin (HBB) signal and lower mitochondrial and ribosomal read fractions, consistent with reduced background and stress-associated transcriptional signatures.

In addition to analytical performance, Evercode WT v4 provides a scalable, instrument-free workflow enabled by combinatorial barcoding and fixation-based sample processing. Fully supported data analysis through the Parse Biosciences pipeline and Trailmaker platform streamlines data interpretation.

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More Cells, More Samples, More Clarity

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