APPLICATION NOTE
EVERCODE WHOLE TRANSCRIPTOME

A Researcher's Head-to-Head Evaluation of Evercode™ and Chromium™ in PBMCs

Comparison Highlights

- Customer-focused study evaluating technologies for a long term project.
- Robust performance in gene expression profiles with both technologies.
- Significant reduction in cell stress with Evercode WT v2.

INTRODUCTION

Single cell RNA-sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) can provide valuable insights into the etiology of autoimmune diseases.

Dr. Howard Davidson coordinated a head-to-head comparison of two of the leading scRNA-seq technologies: Chromium Next GEM Single Cell 3' Kit v3.1 (10x Genomics) and Evercode WT v2 (Parse Biosciences).

Data contributed by:

Howard Davidson, PhD

Associate Professor

University of Colorado
Anschutz Medical Campus

Dr. Davidson evaluated scRNA-seq technologies across performance and ease of use for a large cohort study to span 5 years. He aims to improve prediction of disease progression in type 1A diabetes.

METHODS

Sample Processing

Dr. Davidson cryopreserved PBMCs from a healthy donor and split into two aliquots. One aliquot was fixed with Evercode Cell Fixation v2 and sent to Parse Biosciences for barcoding and library preparation using Evercode WT v2. The other aliquot was sent to the Genomics Shared Resource Facility at University of Colorado Anschutz Medical Campus (Genomics Core at CU Anschutz) for library preparation using a Chromium Next GEM Single Cell 3' Kit v3.1 (3' v3.1) (Figure 1).

Sequencing and Data Analysis

Libraries from both technologies were sequenced by the Genomics Core at CU Anschutz on an Illumina® Novaseq™ 6000. FASTQ files were then downsampled to the same read depth. The Evercode WT v2 data were analyzed with the Parse Biosciences analysis pipeline v1.0.3. The 3' v3.1 data were analyzed with Cell Ranger™ v7.0.1 with intron mode enabled.

Data Filtering and Cell Type Annotation

To prevent potential bias, low-quality cells were removed by filtering raw output matrices with the same quickPerCellQC method, and then downsampled to 4,852 cells. The datasets were integrated with Seurat v4.3.0 and clustered. Both Azimuth and Monaco references were used to enhance cell annotation accuracy.

Reduced Cell Stress

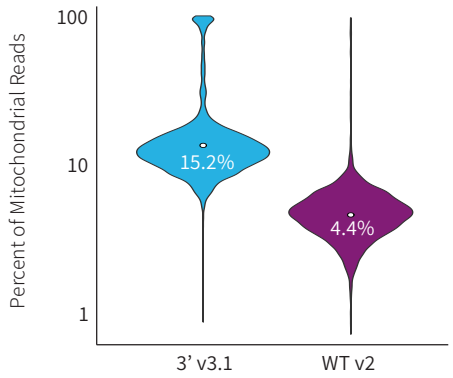


Figure 2. Mitochondrial Transcript Metrics. The percentage of mitochondrial reads detected as a proportion of total reads per experiment before data filtering.

RESULTS

Comparing Mitochondrial RNA Proportions

High mitochondrial read mapping is a quality metric indicating cellular stress and ambient RNA levels during sample processing. In droplet-based scRNA-seq, intact mitochondria from stressed and lysed cells can contaminate droplets, impacting rare cell detection and increasing sequencing costs. After filtering poor quality cells, the 3' v3.1 assay lost 10.2% of cells, while Evercode WT v2 removed only 4.5%. The majority of cells lost in the 3' v3.1 assay were due to high mitochondrial reads, with a median percentage of 15.2% and 10.5% pre and post-filtering. In contrast, Evercode WT v2 had significantly lower percentages of 4.4% and 3.8%, respectively (Figure 2).

EXPERIMENTAL DESIGN

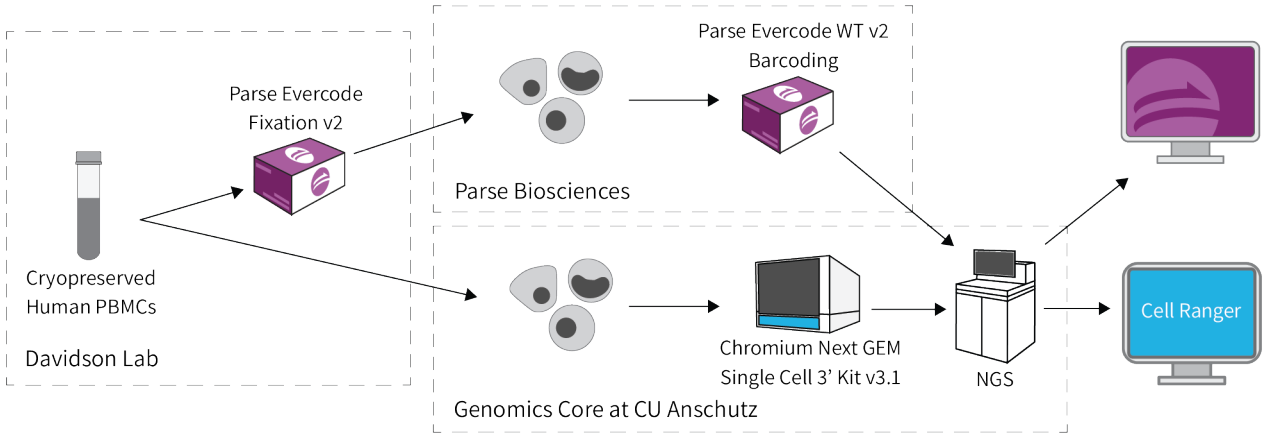
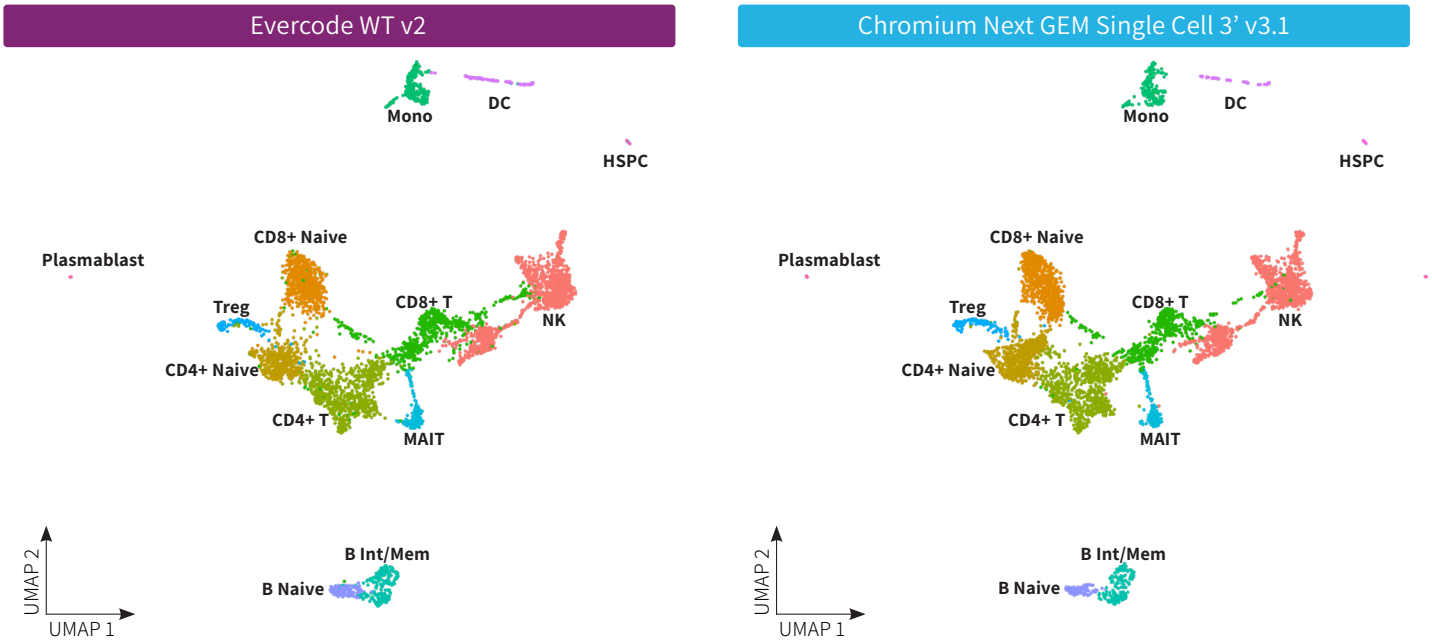


Figure 1. Experimental Design. PBMCs from a healthy donor were cryopreserved. An aliquot of PBMCs fixed by Dr.Davidson was processed by Parse Biosciences with Evercode WT v2, while another aliquot was processed by Genomics Core at CU Anschutz with Chromium Next GEM Single Cell 3' v3.1. Both libraries were sequenced by Genomics Core at CU Anschutz.

Consistent Gene Expression

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



B. Comparison of relative abundance of cell types

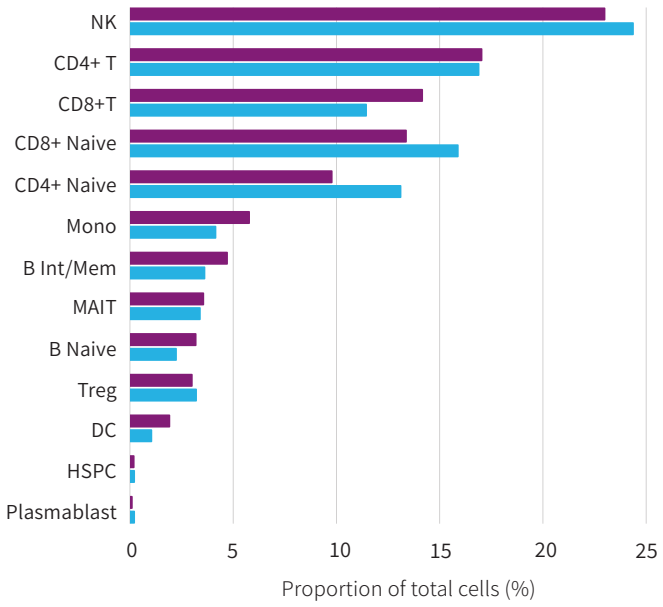


Figure 3. Gene Expression Profile Comparison.
(A) 4,852 human PBMC cells from each Chromium Next GEM Single Cell 3' Kit v3.1 and Evercode WT v2 technology were integrated, clustered with Seurat, annotated, and visualized as UMAPs. (B) A comparison of relative abundance of the major cell types (presented as % of total cells) confirmed concordance of expression between the technologies.

Cell Clustering and Proportions

The integration, clustering, and annotation of data obtained from Evercode WT v2 and 3' v3.1 showed concordant clustering (Figure 3A). Further analysis of cell type proportions found a high degree of similarity across the technologies (Figure 3B).

Sensitivity

In this comparison, Evercode WT v2 captured an average of 11% more genes at 30,000 reads/cell (Figure 4). Increased sensitivity enables better detection of lowly expressed genes, resulting in more comprehensive annotation of cell types.

Sensitive Gene Detection

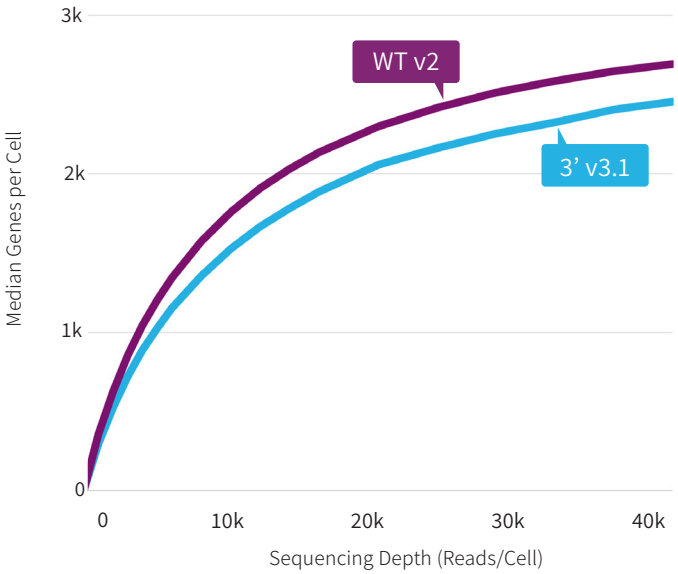


Figure 4. Gene Detection Comparisons. Median genes detected per cell across different sequencing depths for human PBMCs.

CONCLUSION

In an assessment with a researcher's PBMC sample, two leading scRNA-seq products were compared: the 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 and Parse Biosciences Evercode WT v2.

Evercode WT v2 outperformed the Chromium Next GEM Single Cell 3' Kit v3.1 reducing the fraction of mitochondrial reads by 3.5 fold. Both technologies detected similar numbers of genes and cell type proportions. These data confirmed Dr.Davidson's belief that Evercode WT v2 provides a cost effective alternative to Chromium 3' v3.1 for scRNA studies.

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 WT Cell Fixation v2 Up to 4 samples	ECF2001

We Love Hearing from You

Website: parsebiosciences.com

Email: info@parsebiosciences.com

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