TECHNICAL NOTE
EVERCODE WHOLE TRANSCRIPTOME

Comparison of Evercode™ WT v2 and Chromium™ Next GEM Single Cell 3' Kit v3.1 in Mouse Lymph Node Nuclei

INTRODUCTION

A head-to-head evaluation of single cell RNA-seq technologies was performed between droplet-based microfluidics (10x Genomics™ Chromium Next GEM Single Cell 3' Kit v3.1) and combinatorial barcoding (Parse Biosciences Evercode WT v2). The evaluation was conducted using nuclei from mouse lymph nodes, a complex tissue type with a diverse population of immune cells.

Comparison Highlights

- Head-to-head sensitivity comparison shows increased gene detection.
- Integrated results show consistent gene expression and cell type proportions.
- More differentially expressed genes detected between every cell type.

EXPERIMENTAL DESIGN

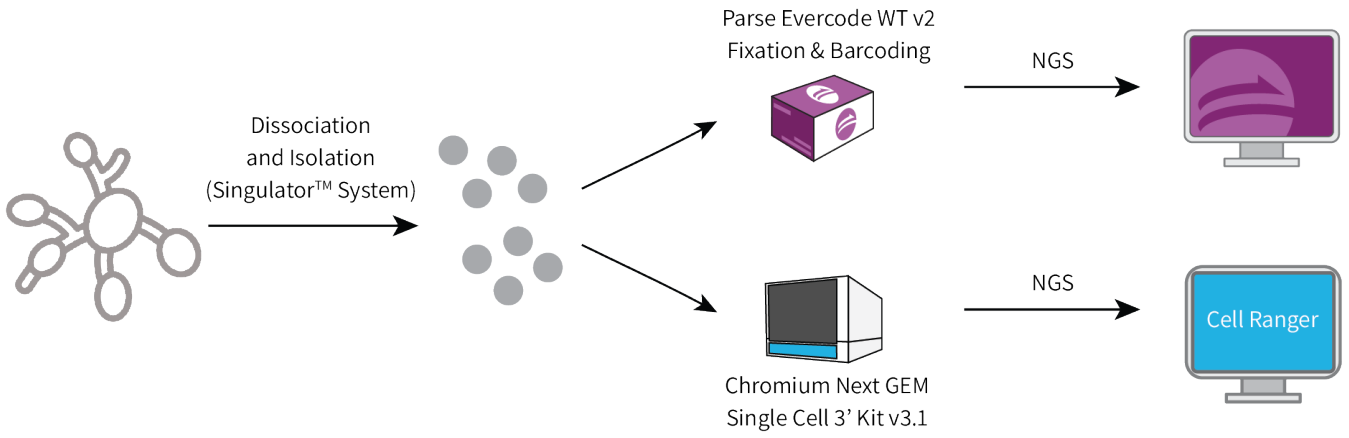


Figure 1. Experimental Design. Mouse lymph node tissue was dissociated into a single nuclei solution with a Singulator™ workflow (S2 Genomics). The samples were strained, centrifuged, and resuspended in S2 Genomics Nuclei Storage Buffer and RNase Inhibitor. The sample was split, and half of the sample 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. The sequencing data were processed with each manufacturer's respective analysis pipeline.

More Genes, Less Sequencing

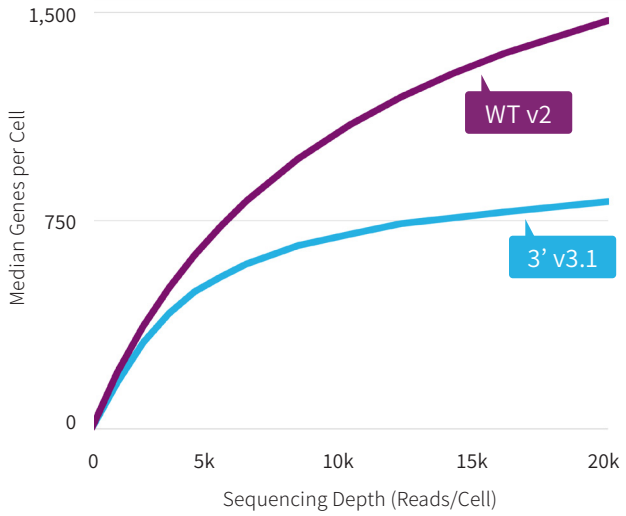


Figure 2. Gene Detection. Median genes detected per nuclei across different sequencing depths for mouse lymph node tissue.

METHODS

Sample Collection

Fresh lymph node tissue, two inguinal and two brachial nodes, were collected from an adult CD-1 mouse and immediately processed. After isolation with the Singulator™ 100 (S2 Genomics), nuclei were strained, centrifuged, and resuspended in S2 Genomics Nuclei Storage Buffer & RNase Inhibitor.

10x Genomics Chromium Next GEM 3' v3.1

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 vendor procedures.

Parse Biosciences Evercode WT v2

The nuclei were fixed with Evercode Nuclei Fixation v2. Whole transcriptome sequencing libraries were prepared using Evercode WT v2.

Sequencing and Data Analysis

Both the 10x Genomics and the Parse Biosciences libraries were sequenced on an Illumina© Nextseq™ 550 High Output Kit v2.5 (150 Cycles). The 10x Genomics data were analyzed with Cell Ranger™ v7.0.1 with intron mode enabled, and the Parse Biosciences data were analyzed with the Parse Biosciences analysis pipeline v1.0.5.

All libraries were sequenced to similar number of reads. To remove low quality cells, pipeline outputs were filtered using the quickPerCellQC method. The Evercode WT v2 and 10x Genomics 3' v3.1 datasets were integrated with the Seurat v4 fast integration method (rPCA). 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.

RESULTS

Sensitivity

Improved sensitivity enhances the detection of lowly expressed genes, leading to a more thorough cell type annotation. At a read depth of 20,000 per cell, 82% more genes were detected in the case of Evercode WT v2 compared to the Chromium Next GEM Single Cell 3' Kit v3.1 in this analysis (Figure 2).

Assessing On Target Reads

Transcripts mapping to mitochondrial and ribosomal proteins typically are not informative to underlying biology and add unnecessary costs by wasting sequencing reads. The median percent of reads mapping to mitochondrial or ribosomal proteins in Evercode WT v2 was over ten times lower compared to the median percent in the 3' v3.1 assay (Figure 3).

More Useful Reads in Cells

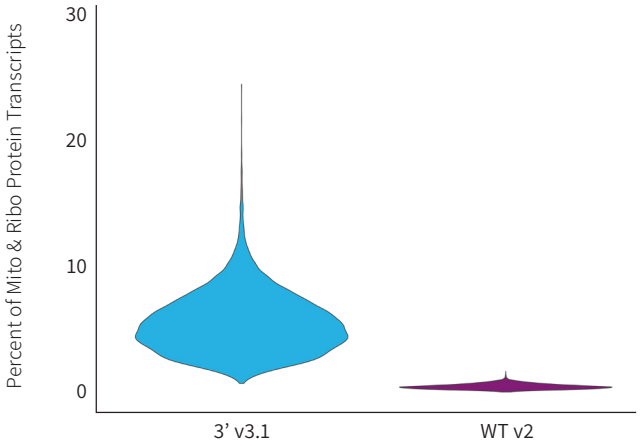
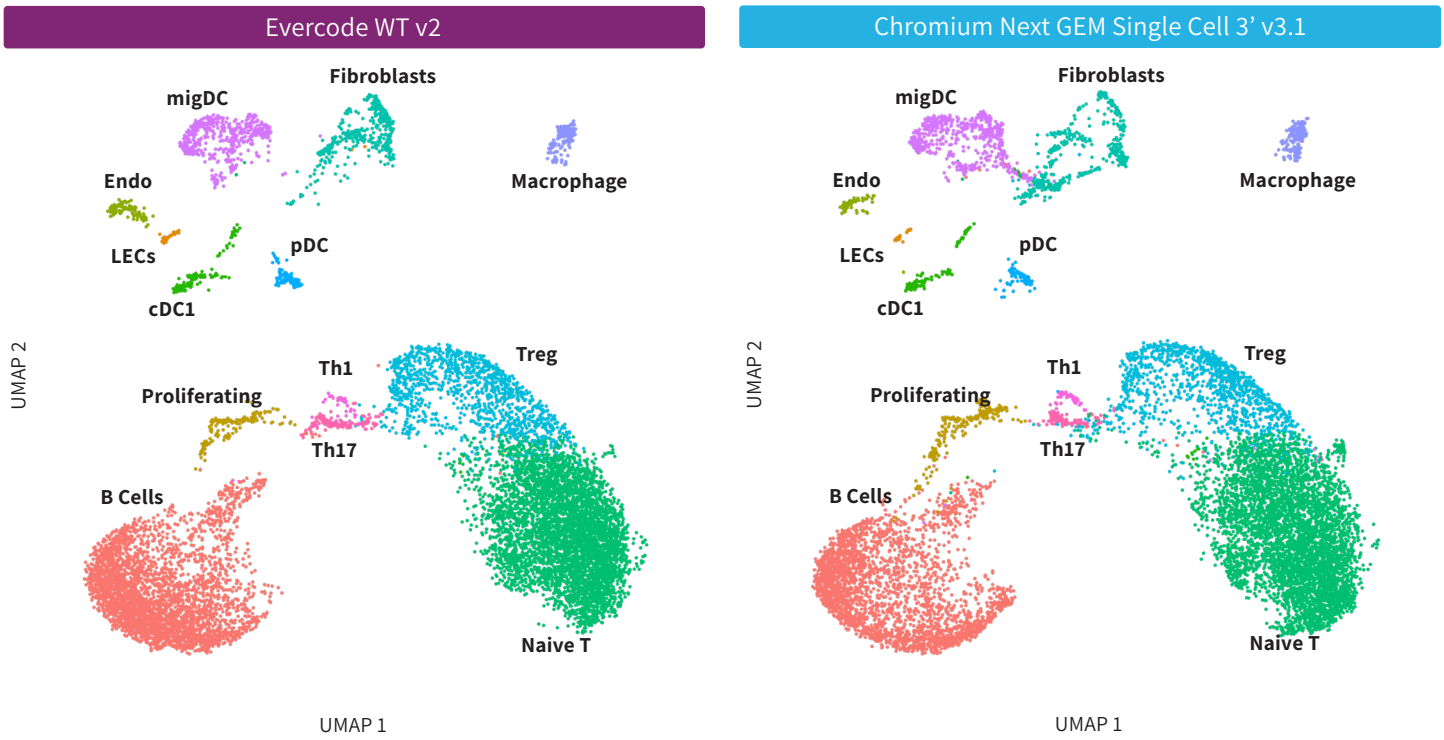


Figure 3. Mitochondrial and Ribosomal Protein Transcripts. The percentage of mitochondrial and ribosomal protein reads detected as a proportion of total reads for all cells.

Integrated Results Confirm Gene Expression Profiles

A. Clustering of integrated Evercode WT v2 and Chromium Next GEM Single Cell Kit v3.1 mouse lymph node nuclei scRNA-Seq



B. Comparison of relative abundance of cell types

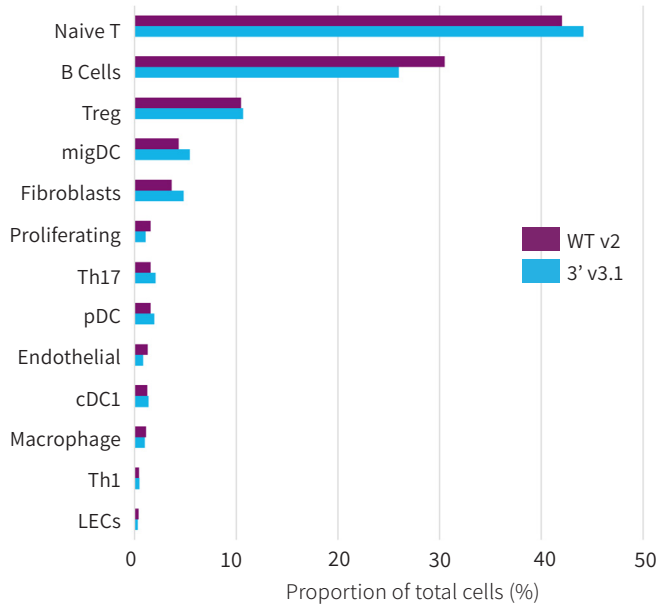


Figure 4. Gene Expression Profile Comparison. (A) 11,795 nuclei from Evercode WT v2 and 11,050 nuclei from Chromium Next GEM Single Cell 3' Kit v3.1 were integrated, clustered, annotated with Seurat, and visualized separately in annotated UMAPs. (B) A comparison of relative abundance of the major cell types (presented as % of total cells) was performed to confirm concordance of expression between the technologies.

Additionally, reads that can't be assigned to any valid cells contribute to wasted resources and data. In this evaluation, 84.7% of reads were assigned to valid cells with Evercode WT, whereas 3' v3.1 achieved 68.1%. A separate study observed similar patterns with Evercode WT at 84.2% and 3' v3.1 at 50.8% reads in cells (Brown et al, 2023).

Cell Proportions and Differential Expression

Integration of the data from Evercode WT v2 and 3' v3.1 resulted in highly concordant clustering and cell proportions (Figure 4), indicating both technologies capture cell types without bias. Investigation of the number of differentially expressed (DE) genes for each cell type showed that Evercode v2 consistently detected more DE genes

Increased Detection of Cell Markers

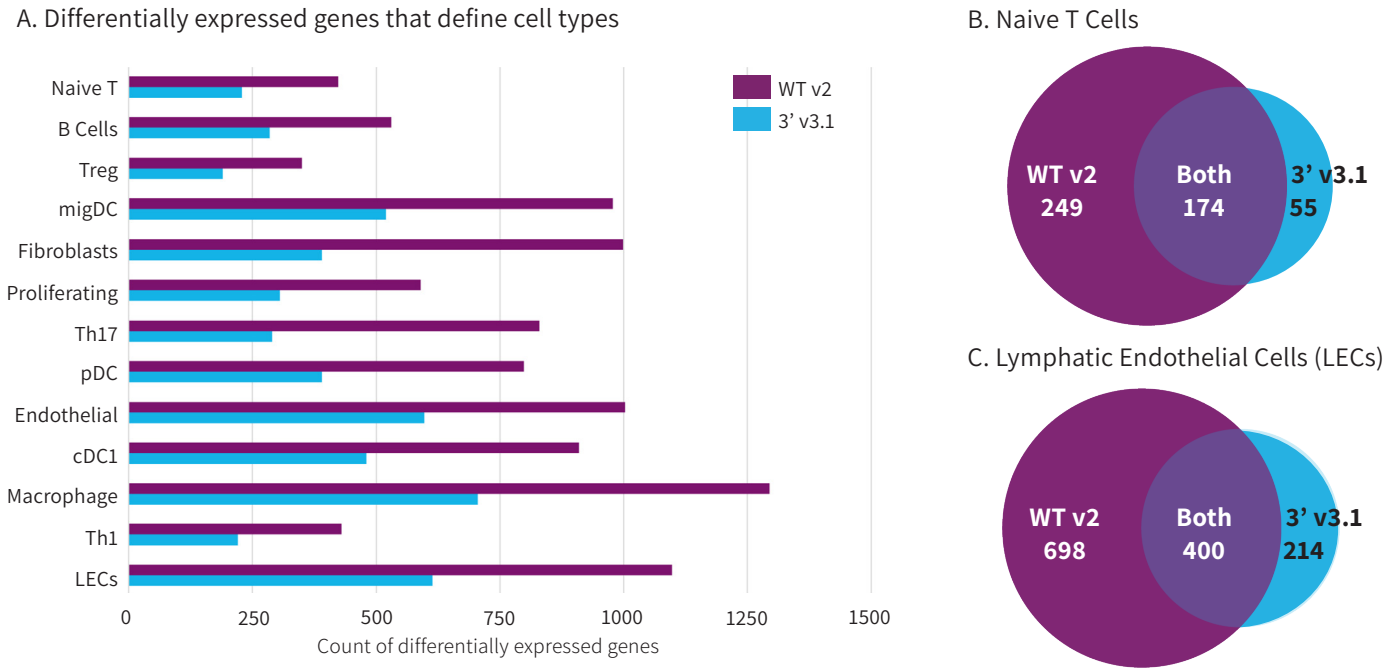


Figure 5. Comparison of Differentially Expressed Genes. (A) The number of differentially expressed genes for each major cell type, ordered by abundance of the cell type. The uniqueness of differentially expressed genes in the highest (B, Naive T cells) and lowest (C, LECs) abundant cell types were further 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.

than Chromium Next GEM 3' v3.1 across all cell types (Figure 5). Notably, the differential genes detected by Evercode v2 largely overlapped with those detected by 3' v3.1, highlighting that the increased number of differential genes primarily results from Evercode v2's higher sensitivity rather than technological differences. In the case of Naive T cells, the most abundant cell type, Evercode WT v2 identified 85% more DE genes than 3' v3.1. For the least abundant cell type, Lymphatic Endothelial Cells (LECs), Evercode WT data showed 79% more DE genes.

CONCLUSION

In the evaluation, the Evercode WT v2 outperformed the 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 in gene detection at all sequencing depths.

Both technologies recovered similar cell types proportions. Evercode WT v2 also detected significantly more differentially expressed genes across all cell types. Overall, Evercode WT v2 demonstrated higher sensitivity and provided more information per nuclei in mouse lymph nodes compared to Chromium Next GEM 3' v3.1.

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