TECHNICAL NOTE
EVERCODE WHOLE TRANSCRIPTOME

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

Comparison Highlights

- Pronounced difference in gene sensitivity from the head-to-head comparison
- Ambient RNA contamination is significantly reduced by not using droplets
- Higher sensitivity and reduced ambient RNA contamination improves cell cluster resolution

INTRODUCTION

A head-to-head evaluation of single cell RNA-seq technologies was performed between a droplet-based microfluidics approach (10x Genomics™ Chromium Next GEM Single Cell 3'Kit v3.1) and a combinatorial barcoding solution (Parse Biosciences Evercode WT v2). Cells from the kidney, an organ containing a complex mix of epithelial, endothelial, immune, and interstitial cell types, were chosen for an in-depth evaluation of cell type resolution and ambient RNA contamination.

EXPERIMENTAL DESIGN

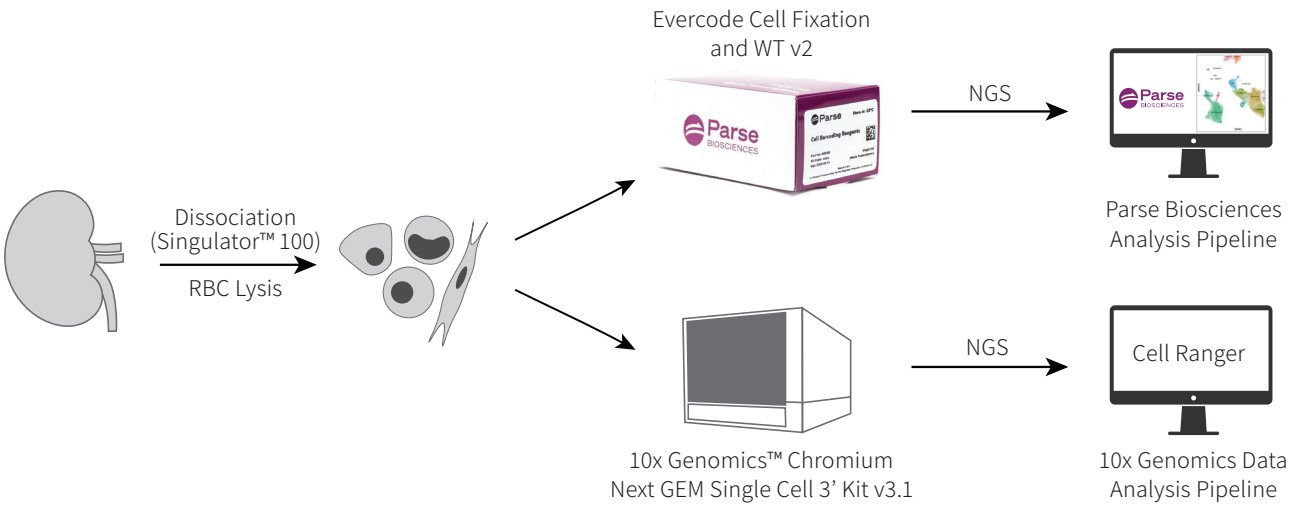

Figure 1. Experimental Design. Mouse kidney tissue was dissociated into a single cell solution with a Singulator 100 (S2 Genomics). The samples were strained and red blood cells (RBCs) were lysed. The sample was split, and half of the sample was prepared with the 10x Genomics Next GEM Single Cell 3' 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.

More Genes, Less Sequencing

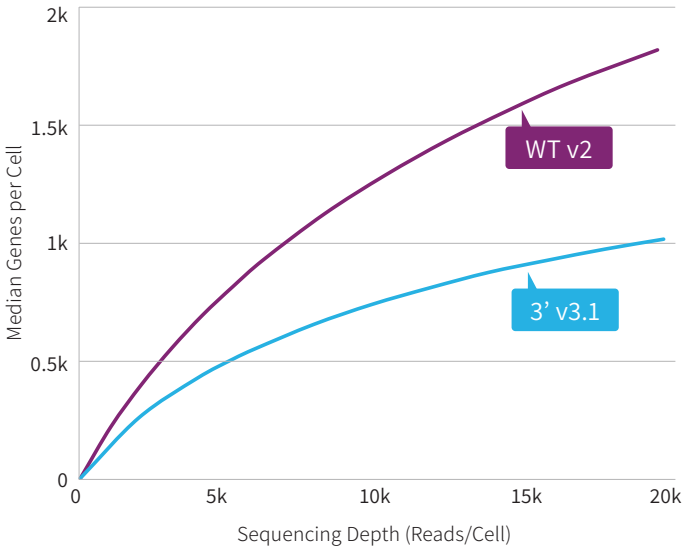

Figure 2. Gene Detection. Median genes detected per cell across multiple sequencing depths in mouse kidney cells. The same sample was split and processed using 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 and Parse Biosciences Evercode WT v2 technologies. The libraries were analyzed by their respective manufacturer's data analysis pipeline.

METHODS

Sample Processing

Kidney tissue was collected from a 6-week old CD-1 mouse, and 220 mg of fresh tissue was immediately processed with the S2 Genomics Singulator 100 using the Kidney Mouse Cell Isolation protocol and Mouse Kidney Cell Isolation Reagent (S2 Genomics, #100-064-631). Red blood cells (RBCs) were lysed with RBC Lysis Buffer from G-Biosciences. Cell counts and viability were determined (Nexcelom K2), and half of the sample was processed using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 to partition and prepare sequencing libraries, and the other half was fixed using the Evercode Cell Fixation v2 kit. Tissue harvesting, dissociation, GEM library preparation, and Evercode Cell Fixation v2 were performed at an independent laboratory. Fixed cells were then shipped to Parse Biosciences for barcoding and library preparation with Evercode WT v2.

Sequencing and Data Analysis

Both libraries were sequenced on an Illumina Novaseq™ 6000 by the same third-party service provider. 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 v1.0.2 analysis pipeline. Each dataset was analyzed independently without applying any additional quality control filtering beyond each manufacturer's default pipeline. Both datasets were downsampled to 9,256 cells and 18,898 mean reads per cell and independently clustered with Seurat v4.0. Clusters were manually annotated using marker genes described in the literature (Balzer et al., Annu Rev Physiol. 2022 Feb 10;84:507-531).

RESULTS

Sensitivity

Increased sensitivity enables better detection of lowly expressed genes, resulting in more comprehensive annotation of cell types. In this comparison in mouse kidney cells, the Evercode WT v2 detected 79% more genes than Chromium Next GEM 3' v3.1 at 19k reads per cell (Figure 2).

Comparing RBC and Ambient RNA Contamination

Annotation of the two datasets showed a large cluster of what appears to be RBCs in the Chromium Next GEM 3' v3.1 data that is absent in Evercode WT v2 data (Figure 3). This cluster represented about 8% of total cells and was dominated by hemoglobin genes. Beyond this cluster, hemoglobin contamination was pervasive across the Chromium Next GEM 3' v3.1 dataset (44% of cells had >1% transcripts mapping to hemoglobin), while this contamination was completely absent from Evercode WT v2 (no cells had >1% hemoglobin). Figure 3 highlights the detection of hemoglobin genes Hba-a1 and Hbb-bs across all clusters in the Next GEM 3' v3.1 data. In addition, the Cell Ranger report (Next GEM 3' v3.1 data) describes a low fraction of reads assigned to cells, with high

ambient RNA as a highlighted cause. In contrast, the Evercode WT v2 data had a higher fraction of reads assigned to cells (87%). The pervasive detection of hemoglobin transcripts suggests that lysed RBCs are the substantial source of ambient RNA contamination in the Chromium Next GEM 3' v3.1 data. Conversely, ambient RNA contamination is not evident in the Evercode WT v2 data.

Ambient RNA is a common issue in droplet-based single cell RNA-seq and can introduce a variety of issues, including poor clustering resolution and spurious cell type identification. Ambient RNA is cell-free RNA that often originates from cell lysis during sample preparation. It can be encapsulated in both full and empty droplets, resulting in inaccurate assignment of transcripts to cells and empty droplets being called as cells. Unlike droplet-based methods, Evercode technology is inherently less susceptible to ambient RNA contamination by using cells rather than droplets as the reaction vessel and including a wash step to physically remove cell-free molecules that may be floating in solution.

Reduced Ambient RNA Contamination in Evercode WT

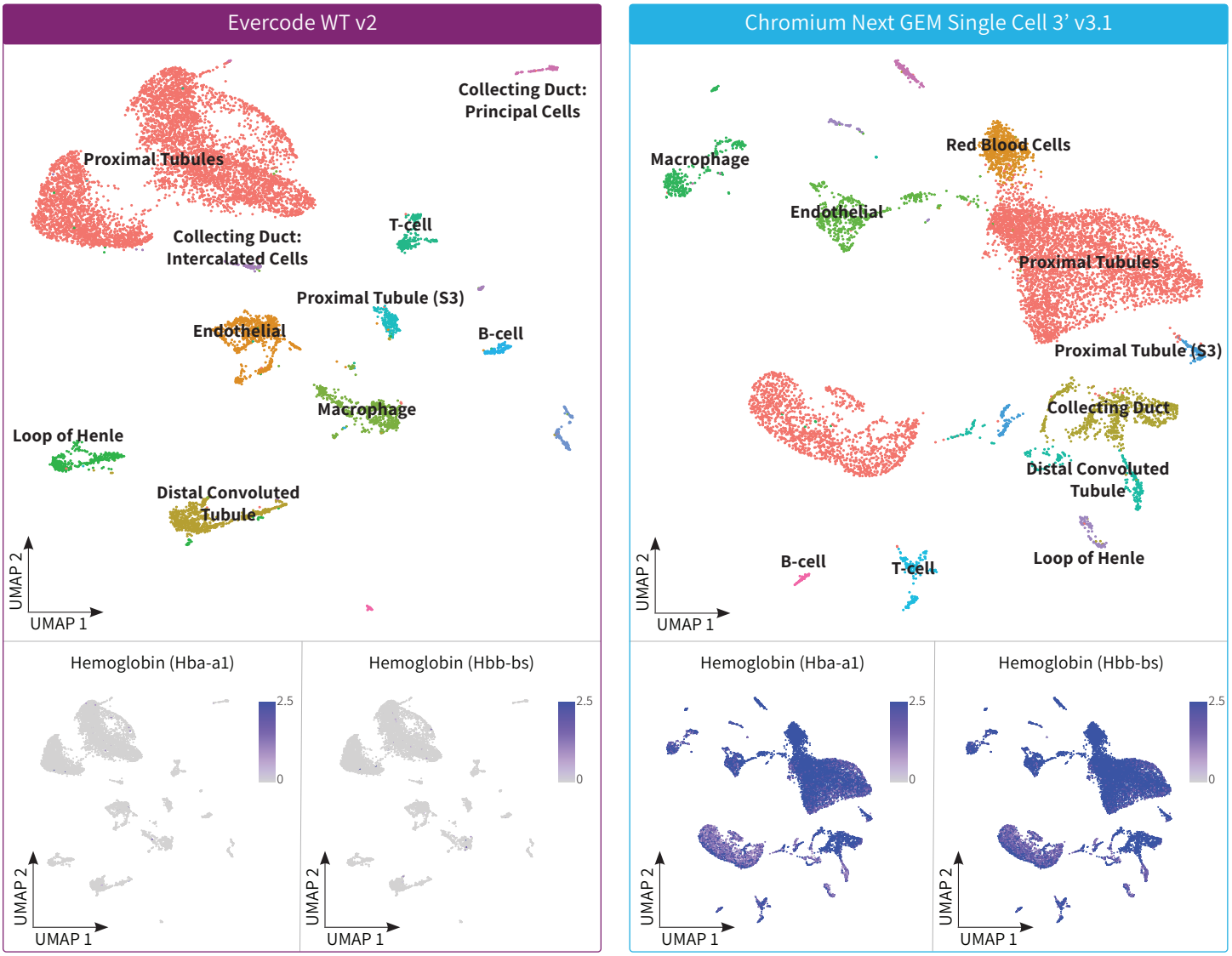

Figure 3. Clustering and Hemoglobin Expression Comparison. 9,256 mouse kidney cells from each Chromium Next GEM Single Cell 3' Kit v3.1 and Evercode WT v2 technology were independently clustered with Seurat, annotated, and visualized as UMAPs. Expression of hemoglobin alpha, adult chain 1 (Hba-a1) and hemoglobin, beta adult s chain (Hbb-bs) are shown for both technologies.

Higher Resolution Cell Clustering

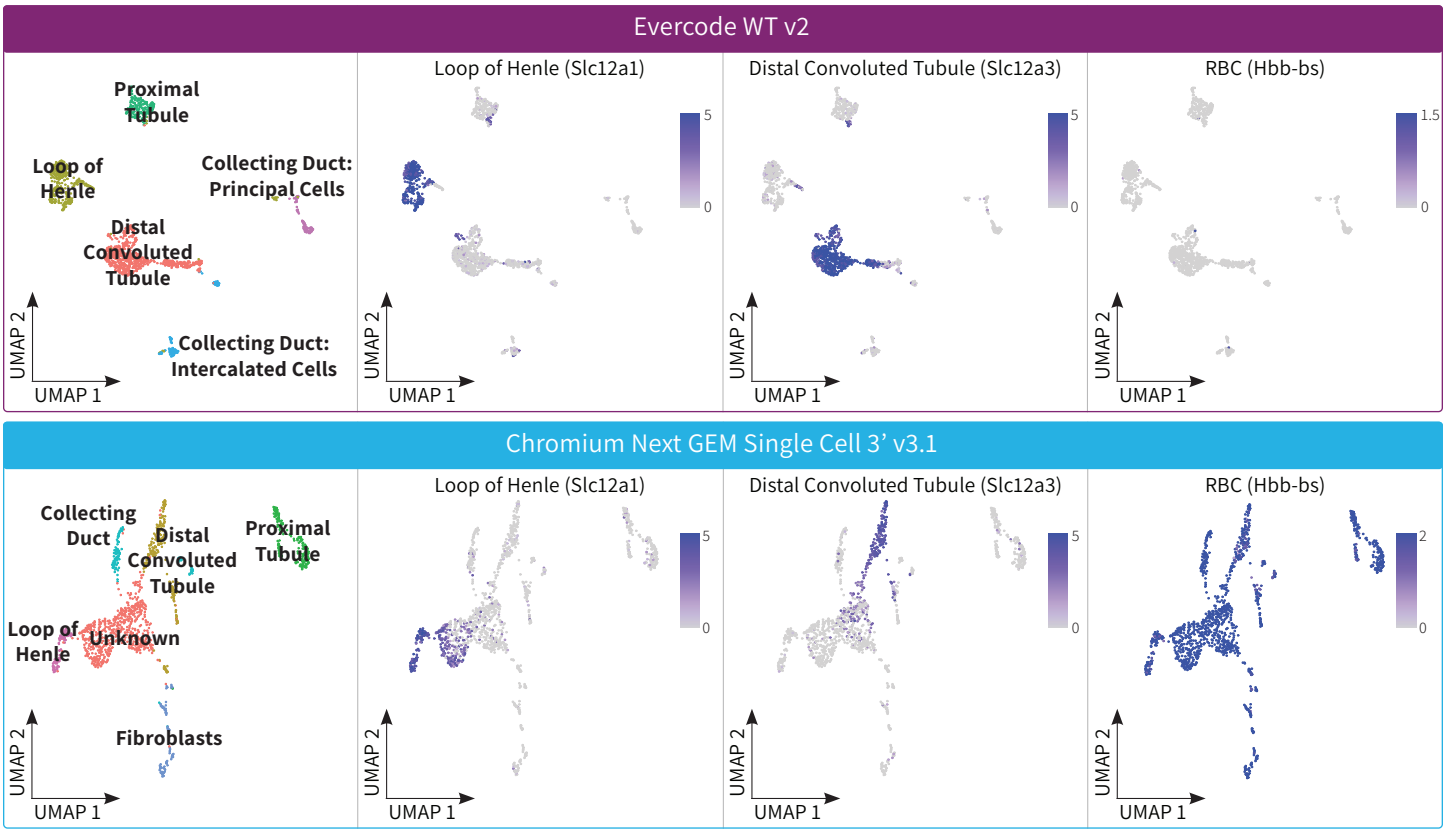

Figure 4. Cell Subtype Cluster Comparison. Clustering of minor tubule kidney-specific cell types in 1,314 cells from Chromium Next GEM Single Cell 3' Kit v3.1 and 1,498 cells from Evercode WT v2. Canonical markers are shown for Loop of Henle (Slc12a1), Distal Convoluted Tubule (Slc12a3), and RBCs (Hbb-bs).

Subcellular Resolution

To more closely examine subcellular resolution, minor tubules cell were subclustered (Figure 4). In the Evercode WT v2 data, there is clear separation between the clusters and canonical cell type markers - including resolving two types of collecting duct cells while there is a single cluster in the Next GEM 3' v3.1 data. In the Chromium Next GEM 3' v3.1 data, canonical markers for the Loop of Henle and distal convoluted tubules are not well separated, and an unknown cluster appears to contain multiple cell types. This decreased cell type resolution illustrates the negative impact of ambient RNA.

CONCLUSION

We compared the performance of 10x Genomics Chromium Next GEM Single Cell 3' Kit v3.1 and Parse Biosciences Evercode WT v2 in mouse kidney cells. Overall, Evercode WT v2 detected more genes per cell, had substantially less ambient RNA contamination, and demonstrated higher cluster resolution compared to Chromium Next GEM 3' v3.1.

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