

Performance Summary of Evercode™ Whole Transcriptome v2 Products

More biology with high resolution of cell types

INTRODUCTION

Single cell sequencing is revolutionizing traditional genomic research by providing more complete representations of biological systems. When evaluating single cell RNA-Seq approaches, sensitivity and reproducible performance are highly weighted performance characteristics. The updated Evercode Whole Transcriptome version 2 solution provides substantial gains in these important areas.

- **More sensitive**
Increased gene and transcript detection
- **More robust**
Consistent gene detection between samples
- **Unbiased gene expression**
Gene expression is accurate and unbiased

More genes with less sequencing

Evercode Whole Transcriptome v2 kits have demonstrably improved gene detection over the v1 kits. In some cases, up to 79% more unique genes were detected with the same sample.

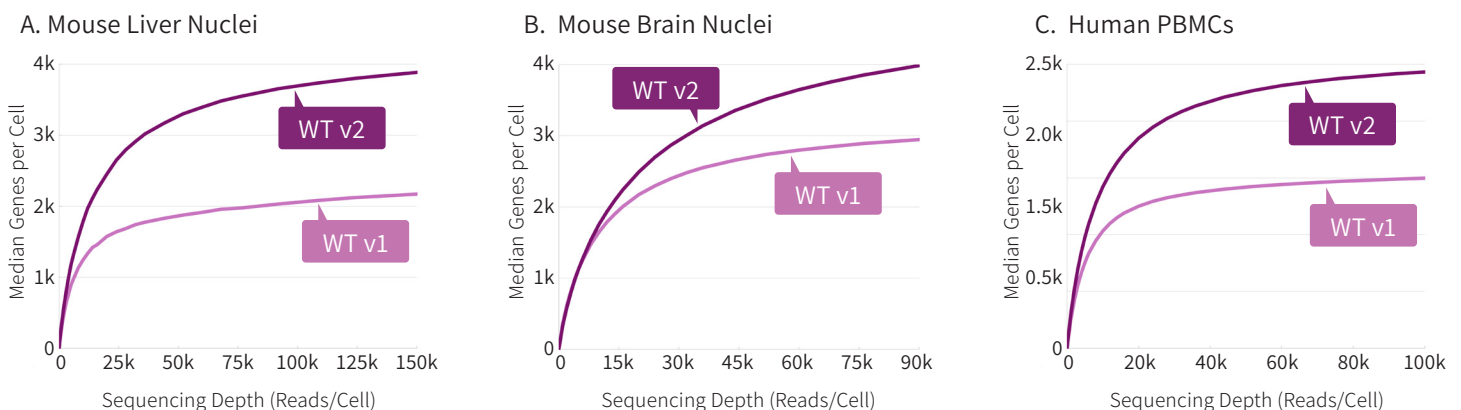


Figure 1. Gene Detection for Multiple Sample Type Comparison. Three sample types, (A) mouse liver nuclei, (B) E18 mouse brain nuclei from two donors, and (C) human PBMCs from four donors, were fixed and prepared with Evercode WT v1 and Evercode WT v2 in parallel. Each experiment was sequenced and processed using the provided data analysis software.

RESULTS

Sensitivity

Higher gene and transcript detection provides researchers deeper biological insights from each experiment.

The Evercode WT v2 solution provided dramatically better sensitivity across all the sample types tested. This result translates across species, regardless of whether the starting material is isolated cells or nuclei (see Figure 1).

In mouse liver nuclei, the WT v2 provided a 79% increase in unique genes detected (150k reads/cell) with a 56% gain at a more common depth of 20k reads/cell. In embryonic mouse brain nuclei, a 37% increase was observed (100k reads/cell) with a 22% gain at 20k reads/cell.

In human PBMCs, the WT v2 detected 63% more unique genes (100k reads/cell) with 48% more genes than WT v1 at a sequencing depth of 20k reads/cell.

Robustness

Consistent gene detection between samples results in fewer sample failures, more predictable sequencing planning, and straightforward data analysis.

Lower variation in gene detection was pronounced and reproducible across sample types. Evercode WT v2 exhibited more consistent gene detection than identical samples prepared with Evercode WT v1 in embryonic mouse brain nuclei (Figure 2A) and human PBMCs (Figure 2B).

Unbiased Gene Expression

Results obtained with Evercode WT v2 are concordant with Evercode WT v1 and flow cytometry, enabling researchers to integrate previously acquired data.

Across all cell types tested, there's no bias in recovery or gene expression with the updated performance of Evercode WT v2 (Human PBMCs in Figure 3).

More consistent gene detection

Sample-to-sample gene detection variability has been reduced in Evercode Whole Transcriptome v2 products - eliminating sample under-representation while increasing predictable outcomes.

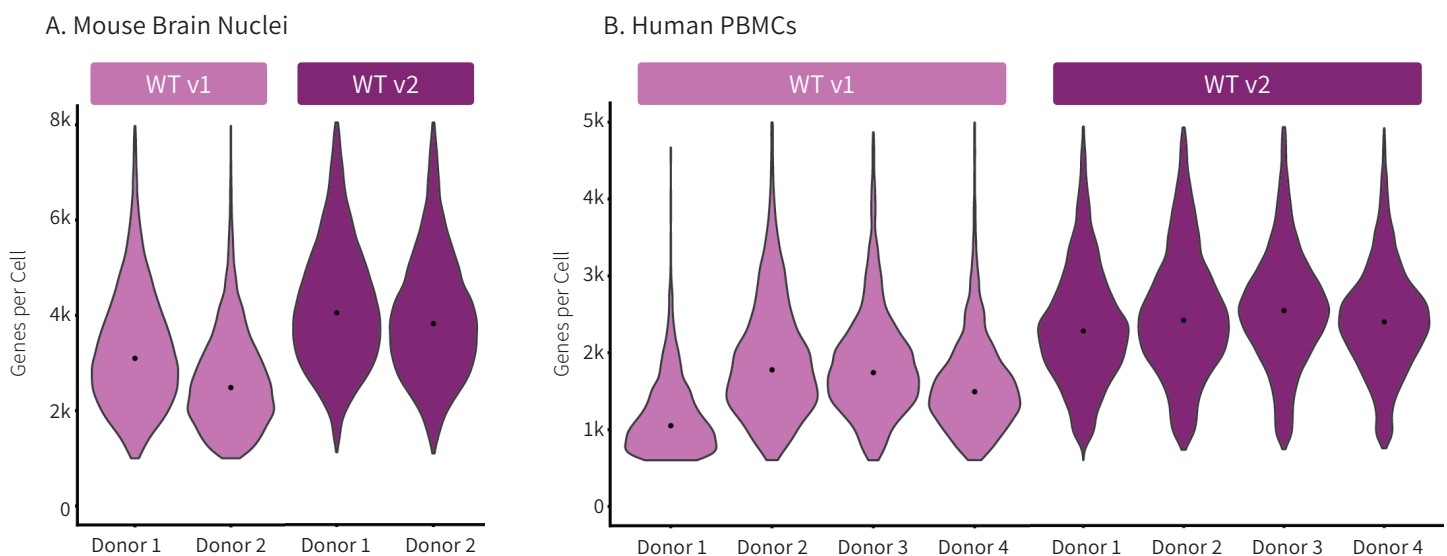
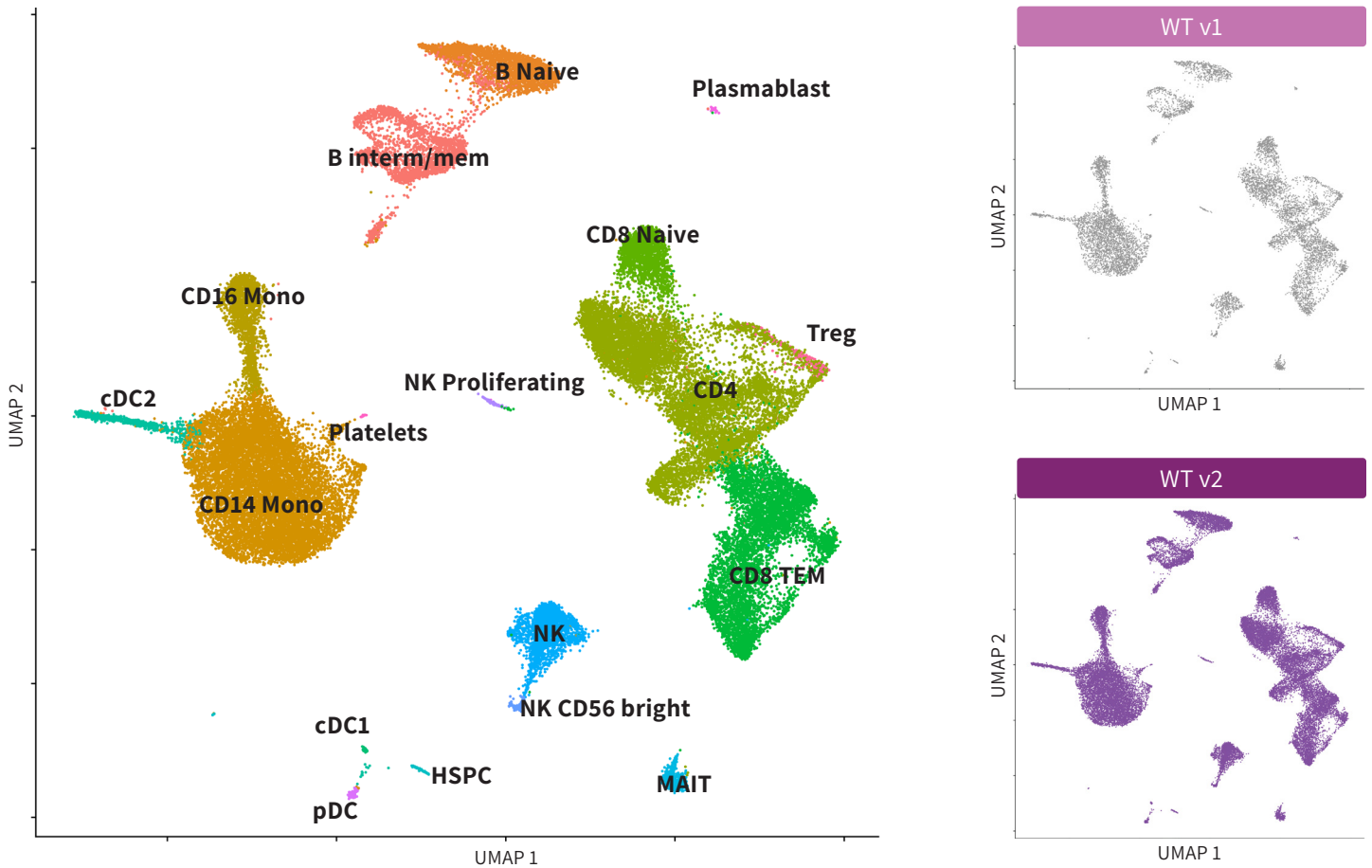


Figure 2. Gene Detection Across Samples. (A) Two embryonic mouse brain samples and (B) samples from four human PBMC donors were prepared in parallel using the Evercode WT v1 and Evercode WT v2 kits. The distribution of genes per cell are plotted for each sample and technology.

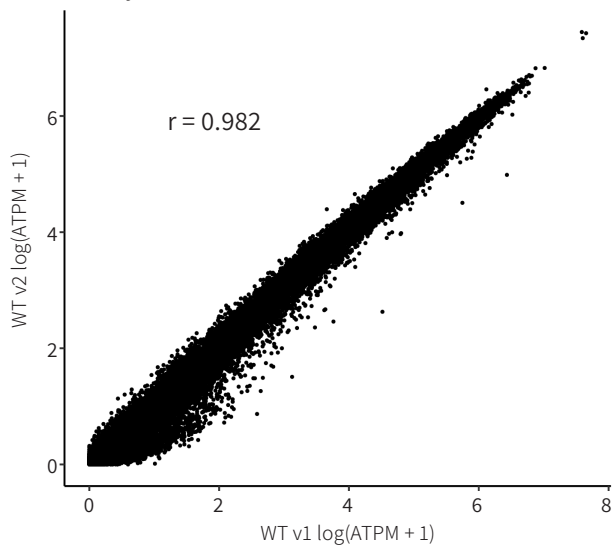
Unbiased gene expression

Researchers can transition to Evercode Whole Transcriptome Solution v2 and benefit from gene sensitivity and improved sample robustness while integrating seamlessly with other studies.

A. Human PBMC clustering of integrated WT v1 and WT v2 data



B. Chemistry version correlation



C. Comparison of relative abundance of cells

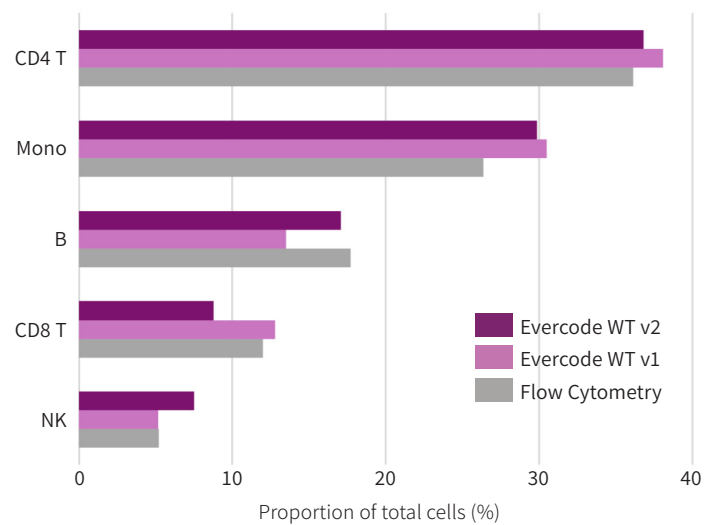


Figure 3. Gene Expression Profile Comparison. (A) Human PBMC results for Evercode WT v1 and Evercode WT v2 were integrated and clustered together and evaluated. (B) The gene expression correlation was confirmed (log average transcripts per million) between the two chemistry versions. (C) A single donor was evaluated for concordance by identifying the major cell types and comparing to flow cytometry results provided by a third party.

METHODS

We fixed four PBMC samples using the Evercode Cell Fixation v1 and v2 kits, in parallel. Two embryonic mouse brain samples and a sample from an adult mouse liver were fixed in parallel with the Evercode Nuclei Fixation v1 and v2 kits.

Sequencing libraries were prepared with the Evercode WT v1 (v1 fixed samples) and Evercode WT v2 (v2 fixed samples), and sequenced.

The sequencing output was analyzed with the Parse Biosciences data analysis pipeline. UMAP plots were generated with clustering analysis in Seurat and cluster annotation in Azimuth.

CONCLUSION

The Evercode Whole Transcriptome v2 products provide substantially better performance over the previous, Evercode Whole Transcriptome v1 products.

The improvements in sensitivity translate to more effective experiments, while the improvements to robustness ensure better results every time. Unbiased gene expression confirms the deeper investigation of the entire biological system, not just a few markers. The Evercode WT v2 uncovers more biology at higher resolution, making single cell insights more accessible to researchers.

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 Cell Fixation v2 Up to 4 samples | ECF2001 |
| Evercode Nuclei Fixation v2 Up to 4 samples | ECF2003 |

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