Pushing the Boundaries of Gene Sensitivity with the Chromium Single Cell Gene Expression v3

Abstract

Single cell sequencing technologies provide a high-resolution view of complex biological systems and processes, revealing molecular mechanisms of development and disease at the level of individual cells. Direct analysis of tissues or cellular populations at the single cell level allows you to characterize intracellular heterogeneity, cell types and states, and dynamic cellular transitions. Here, we introduce an upgraded version of the 10x Genomics Single Cell Gene Expression Solution. We describe the improved performance of the v3 solution on unstimulated peripheral blood mononuclear cells (PBMCs), as well as three complex tissues (glioblastoma, mouse neural tissue, mouse cardiomyocytes).

Highlights

- Up to two-fold increase in median genes per cell detected at equivalent sequencing depths compared to v2
- Improved detection of cells with low RNA content
- Optimized reagents, consumables, and software

Introduction

The ability to measure gene expression from single cells has revolutionized our understanding of developmental biology, tissue heterogeneity, disease progression, and treatment (1-7). Paramount to the success of these single cell RNA sequencing approaches has been the development of robust, sensitive, unbiased, and high-throughput assays that can be applied to a broad range of different cell types. The Chromium Single Cell Gene Expression Solution has been utilized successfully across a variety of species and applications because of its broad applicability, capture rate, throughput, scale, and sensitivity (1-7).

The Chromium Single Cell Gene Expression Solution has been upgraded to version 3 (v3). This improvement includes a substantial increase in sensitivity, with up to a two-fold increase in genes and transcripts detected compared to version 2 (v2). The new Chromium Single Cell Gene Expression Solution maintains a low doublet rate (0.8% per 1,000 cells) and industry-leading high cell recovery efficiency of up to 65%. In addition to improvements to the gene expression assay, the new Chromium Single Cell Gene Expression Solution enables Feature Barcoding technology—allowing for the detection of gene expression and protein abundance, gene expression and CRISPR-mediated perturbations from the same cells and more. (see Documents LIT000030, LIT000032, and LIT000031).

The new Chromium Single Cell Gene Expression Solution also adds new updates to its software tools—Cell Ranger and Loupe Cell Browser. Software updates enable the combined analysis and visualization of gene expression with any barcoded feature including, but not limited to, cell surface proteins and single-stranded gRNAs (sgRNAs). Additionally, the software updates enable the automatic identification of cells in samples containing a large dynamic range of RNA content, resulting in improved detection of low RNA content cells. In addition, it now provides you the ability to perform chemistry batch effect correction when aggregating samples processed on v2 and v3 chemistries to enable more accurate combined analysis.

10x GENOMICS

Methods

Samples

Unstimulated peripheral blood mononuclear cells (PBMCs) (CG00039—Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing), and complex tissues samples—human glioblastoma multiforme (Stage III-B) (prepared using CG00039), C57BL/6 E18 embryonic mouse hippocampus (CG00055—Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing), and fresh pre-dissociated cells from two C57BL/6 E18 embryonic mouse whole hearts were used to prepare single cell suspensions as referenced. The complex tissue samples contained a range of cell sizes, from 4µm up to 15µm.

For each sample type, Chromium Single Cell 3' v2 and v3 libraries with ~1,000 cells targeted were prepared following the protocols outlined in the Chromium Single Cell 3' Reagent Kits v2 User Guide (Document CG00052) and Chromium Single Cell 3' Reagent Kits v3 User Guide (Document CG00183) (Figure 1), respectively. Libraries were sequenced using paired-end sequencing (26bp and 28bp Read 1 for v2 and v3 libraries, respectively, and 91bp Read 2) with a single sample index (8bp) on an Illumina NovaSeq. Samples were sequenced to a depth of > 50,000 raw reads per cell, with raw sequencing data analyzed and visualized with pre-release versions of Cell Ranger 3.0.0 and Loupe Cell Browser 3.0.0.

Chromium Single Cell Expression v3 Shows Increased Sensitivity

Cells originating from the same single cell suspensions were processed with both the Chromium Single Cell Expression Solution v2 and the Chromium Single Cell Expression Solution v3. Using a fixed sequencing depth (50,000 raw reads per cell), samples prepared using v3 chemistry show a 20-68% increase in median genes and 26-71% increase in median UMIs detected relative to v2 samples. Further sampling of sequencing reads at 20,000 raw reads per cell revealed a similar trend with v3 samples showing a 10-46% increase in median genes and 7-37% increase in median UMIs detected per cell relative to v2 (Figure 2 and 3).

Results

When assessing the performance of any single cell solution a critical factor to take into account is the RNA content of the cells tested. The number of genes and UMIs detected varies substantially between samples, owing to differences in RNA content (Figure 2). RNA content can be defined by the number and diversity of RNA transcripts inside a cell. Importantly, detection of these transcripts is dependent on the sequencing depth for a given sample, meaning that one will continue to detect additional genes and UMIs as depth is increased, until the point of saturation. At this point, with each new sequencing read, no new transcripts will be detected. Here we tested a variety of complex samples to show assay

The Chromium Single Cell Gene Expression Workflow

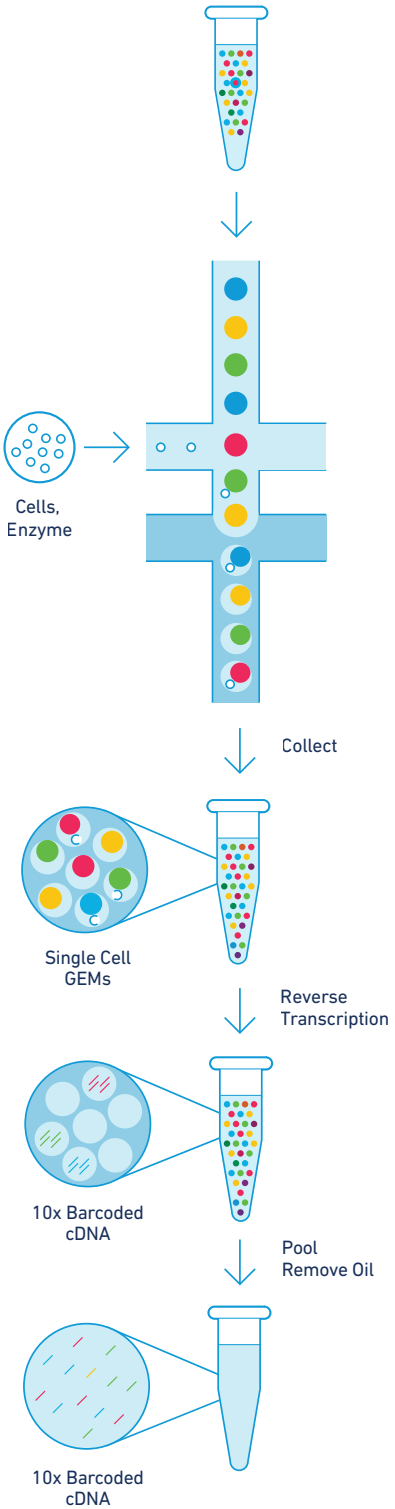

Figure 1. Cell partitioning and barcoded cDNA library preparation was performed on a Chromium Controller with chips and reagents from Single Cell Gene Expression v3 kits. The 10x barcoded gel beads contain Poly(dT) primers for cDNA library preparation. Sequencing libraries are then created from the barcoded cDNA.

performance across a wide range of cell RNA content. For example, with the new v3 chemistry we detected ~3,000 median genes per cell for the glioblastoma sample sequenced at ~50,000 raw reads per cell—a ~20% increase compared to the v2 chemistry. Note that sequencing saturation for this sample was at 42%; at deeper sequencing depths, and thus increased saturation, further improvements in performance between v2 and v3 are seen (Figure 2). Unstimulated peripheral blood mononuclear cells (PBMCs), which have substantially lower RNA content, have only 2,000 genes detected per cell at ~50,000 raw reads per cell (71% sequencing saturation on v3). In conclusion, we detected more median genes per cell at low sequencing depth (~20,000 raw reads per cell) in samples prepared with the new Chromium Single Cell Gene Expression Solution v3 compared to the same samples sequenced at much higher sequencing depth (50,000 raw reads per cell) and prepared with the v2 chemistry.

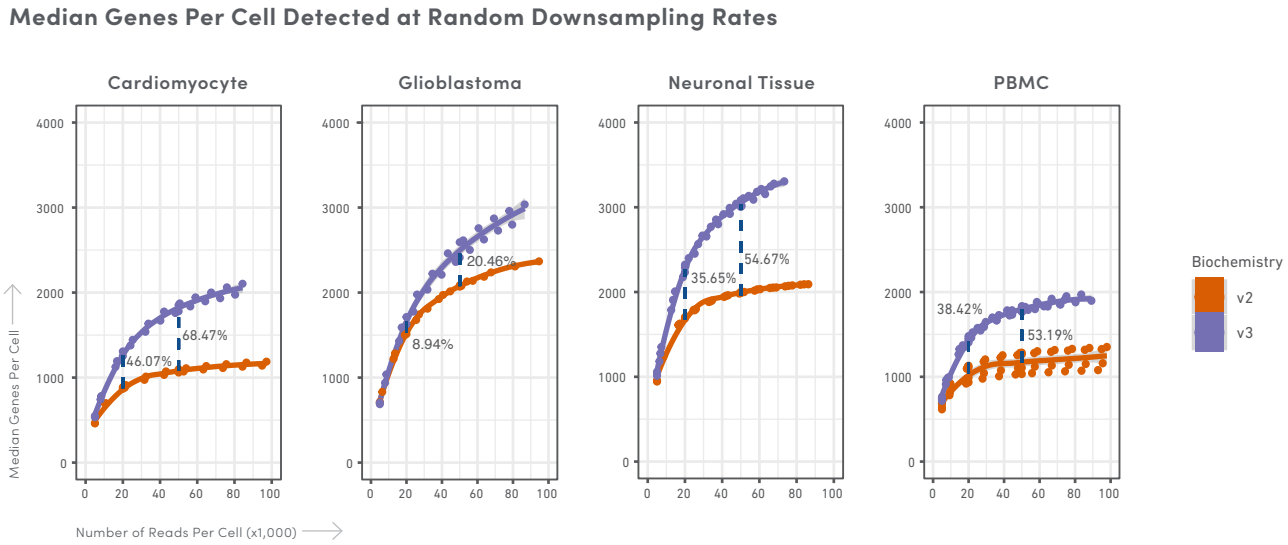

Figure 2. 1,000 cells/experiment. Percentage increased genes/cell provided for each sample at 50,000 and 20,000 reads per cell. Cardiomyocyte and glioblastoma samples run in duplicate, neural tissue samples run in triplicate, PBMC samples run in quadruplicate for both v2 and v3 assays.

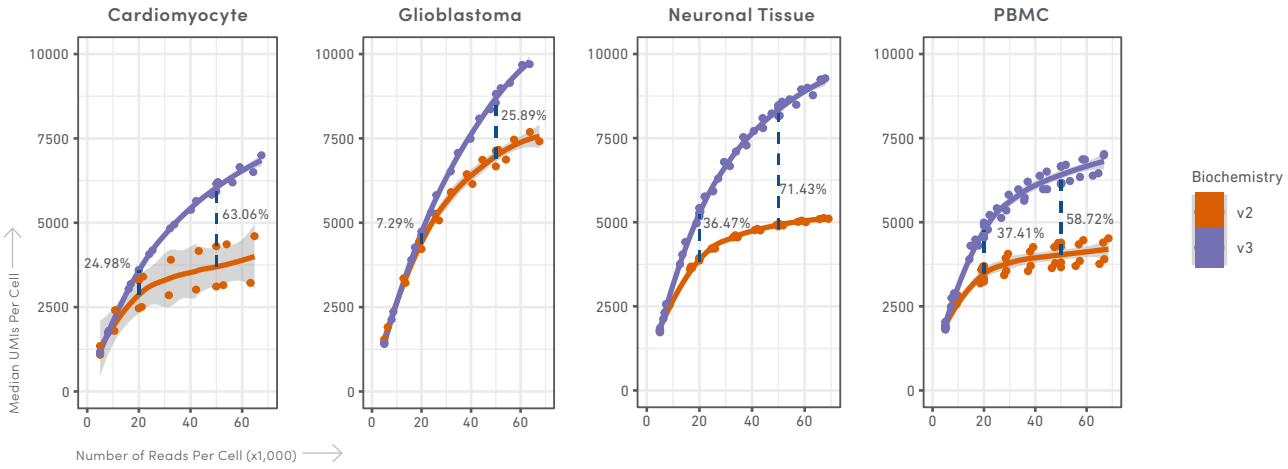

Figure 3. 1,000 cells/experiment. Percentage increased UMIs/cell provided for each sample at 50,000 and 20,000 reads per cell. Cardiomyocyte and glioblastoma samples run in duplicate, neural tissue samples run in triplicate, PBMC samples run in quadruplicate for both v2 and v3 assays.

Gains in v3 Are Additive to Information Available in v2

While sensitivity is increased with the new Chromium Single Cell Gene Expression Solution v3, there is no loss or significant change of information previously available with the v2 solution. When replicate PBMC samples were run following both the v2 and v3 protocols, with sub-clusters annotated based on specific marker genes, the percentages and relative proportion of known cellular populations identified were comparable between both versions (Figure 4,5). As with v2 chemistry, we see minimal evidence of significant technical batch effects on v3 samples (see Document CG000170). Overall clustering profiles between v2 and v3 are highly similar for these samples, where well-known cell types are being annotated (Figure 5).

The improvements inherent to the new v3 chemistry, especially the increased numbers of genes detected, will result in subtle shifts in how cells are projected into tSNE space. Cell Ranger 3.0.0 can, optionally, perform alignment of biologically similar samples produced with both v2 and v3 chemistry in order to better align data produced from the two different versions.

Proportion of Cell Types Detected in Four Replicate PBMC Samples Run on v2 and v3 Solutions

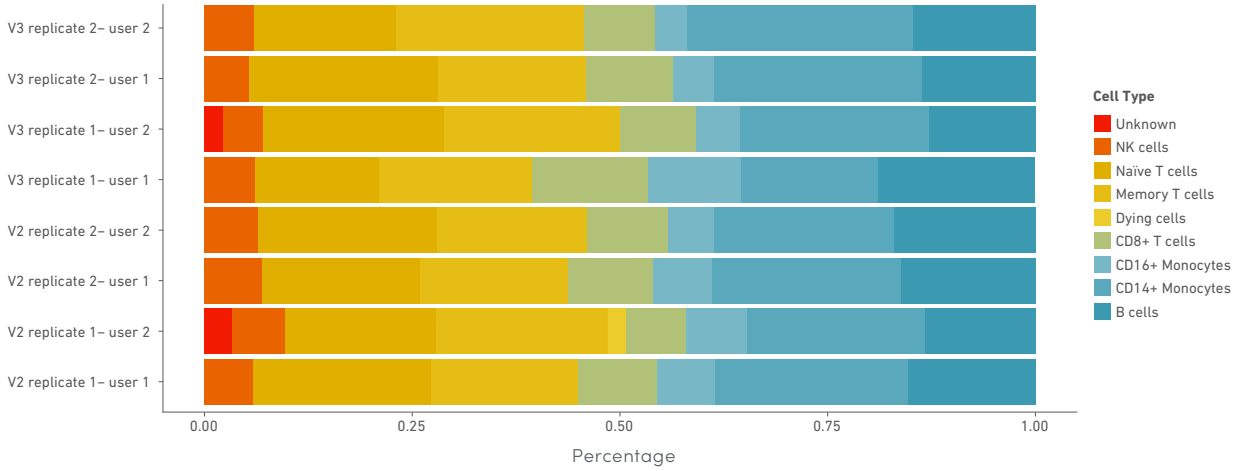

Figure 4. Single cell RNA sequencing data from four replicates of unstimulated PBMC samples run on the v2 and v3 solutions were clustered and manually annotated according to known specific marker genes.

Identical Sample Processed on v2 and v3 Solutions Show Comparable Clustering and Relative Abundance of Major Cell Types

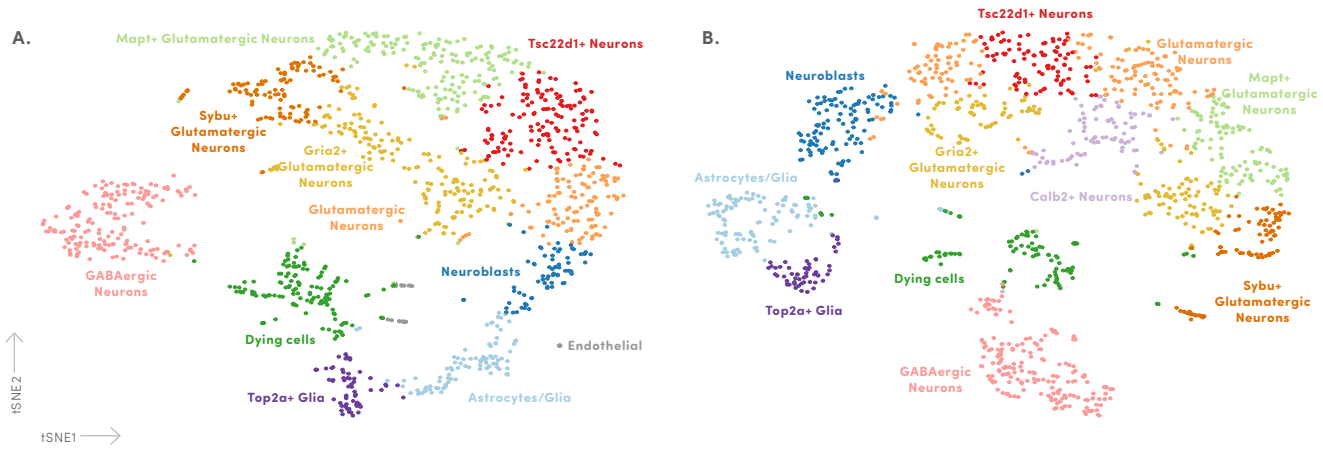

Figure 5. A. Unsupervised clustering and manual annotation of v2 1,000 cell mouse neural tissue data. **B.** Unsupervised clustering and manual annotation of v3 1,000 cell mouse neural tissue data.

Conclusion

In this Application Demonstration, we describe the improved performance of the Chromium Single Cell Gene Expression Solution v3 compared to v2 on unstimulated peripheral blood mononuclear cells (PBMCs) as well as three complex tissues (glioblastoma, mouse neural tissue, mouse cardiomyocytes). We demonstrate that improvements in solution performance lead to increased flexibility in experimental design and can reduce sequencing read requirements while maintaining high gene sensitivities.

Resources

Datasets	go.10xgenomics.com/scRNA-3/datasets
Seminars	go.10xgenomics.com/scRNA-3/seminars
Application Notes	go.10xgenomics.com/scRNA-3/app-notes
Technical Support	go.10xgenomics.com/scRNA-3/support
Publications	go.10xgenomics.com/scRNA-3/pubs

Support

support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588-3260

Legal Notices

For 10x Genomics legal notices visit:
10xgenomics.com/legal-notices

References

1. K.S. Yan, C.Y. Janda, J. Chang, G.X.Y. Zheng, K. Larkin, *et al.*, A Non-equivalence of Wnt and R-spondin ligands during Lgr5+ intestinal stem-cell self-renewal. *Nature* 545, 238-242 (2017)

2. M. Shnayder, A. Nachshon, B. Krishna, E. Poole, A. Boshkov, *et al.*, Defining the Transcriptional Landscape during Cytomegalovirus Latency with Single-Cell RNA Sequencing. *MBio.* 9, e00013-18 (2018)

3. K. Bach, S. Pensa, M. Grzelak, J. Hadfield, D.J. Adams, *et al.*, Differentiation dynamics of mammary epithelial cells revealed by single-cell RNA sequencing. *Nat Commun.* 8, 1-11 (2017)

4. D.T. Montoro, A.L. Haber, M. Biton, V. Vinarsky, B. Lin, *et al.*, A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* 560, 319-324 (2018).

5. M.D. Young, T.J. Mitchell, F.A. Vieira Braga, M.G.B. Tran, B.J. Stewart, *et al.*, Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. *Science* 361, 594-599 (2018).

6. P. Savas, B. Virassamy, C. Ye, A. Salim, C.P. Mintoff, *et al.*, Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nat Med.* 24, 986-993 (2018).

7. P. Savage, A. Blanchet-Cohen, T. Revil, D. Badescu, S.M.I. Saleh, *et al.*, A Targetable EGFR-Dependent Tumor-Initiating Program in Breast Cancer. *Cell Rep.* 21, 1140-1149 (2017).

Resources from 10x Genomics

We are dedicated to helping you get the most out of your 10x Genomics system by offering multiple helpful resources:

Solutions and products

Along with our suite of complete solutions, we offer an ever-growing catalogue of services to help you find the answers to your research questions.

10x Compatible Products

Access our list of key partner products that have been certified compatible to work with our various solutions.

10x University

Immerse yourself in 10x University, a comprehensive step-by-step learning and training environment containing video tutorials and trainings.

Blog

Keep up to date with the 10x Genomics Blog, where you'll find everything from tips and tricks to the latest 10x news.

10x Library

Easy access to our complete library of product literature, customer publications, application notes, protocols, and many other useful resources.

Support

Visit the support site for documentation, software, and datasets that will help you get the most out of your 10x Genomics products.

Contact Us

10xgenomics.com

10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588-3260

+1 925 401 7300
+1 800 709 1208
info@10xgenomics.com

For more locations in US, EU and Asia visit:
10xgenomics.com/company/#locations

Join the 10x Community. Get exclusive access to user forums, blogs, tips and tricks and more. Then, give us your feedback to help make this your community.

- Share your experience
- Read our blog
- Give feedback
- Interact with us

Join our Community at: community.10xgenomics.com

LIT000035 *Rev B Pushing the Boundaries of Gene Sensitivity with the Chromium Single Cell Gene Expression Solution v3*