TECHNICAL NOTE

Chromium Next GEM Single Cell 3' LT v3.1: Reagents, Workflow & Data Overview

Introduction

Chromium Next GEM Single Cell 3' LT v3.1 is a low throughput, cost-effective solution for smaller-scale and pilot studies, which enables profiling whole transcriptome at the single cell level for one hundred to a thousand cells per sample. In combination with Feature Barcode technology, the assay also enables simultaneous cell surface protein detection in single cells. This Technical Note highlights sample preparation, reagents, and workflow specifics for Single Cell 3' LT v3.1, along with information about cell dilution, cell multiplet rate, signal-to-noise data profile, and cluster defining for low cell loads. A comparison of representative data derived from the low throughput Single Cell 3' LT v3.1 assay versus the standard Single Cell 3' v3.1 assay is also presented.

Chromium Next GEM Single Cell 3' LT Workflow

Chromium Next GEM Single Cell 3' LT v3.1 workflow (referred to as low throughput or LT) is similar to the Chromium Next GEM Single Cell 3' v3.1 workflow (referred to as standard), with a few specific updates that are indicated by a "LT" icon adjacent to the updated steps in the Single Cell 3' LT v3.1 User Guides (see Product List & Documents section for link to user guides).

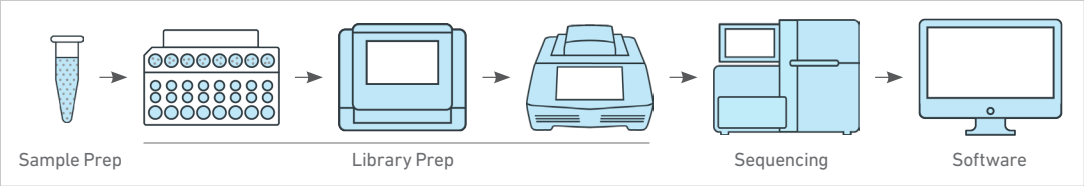


Figure 1. Chromium Next GEM Single Cell 3' LT v3.1 workflow.

Single Cell 3' LT v3.1 assay enables partitioning of 100-1,000 cells per channel of the Chromium Next GEM Chip L for assessing single cell gene expression alone or in combination with cell surface protein expression. Nanoliter-scale Gel Beads-in-emulsion (GEMs) are generated by combining a Master Mix containing cells and enzymes, 10x Barcoded Single Cell 3' LT Gel Beads, and Partitioning Oil onto the chip (Figure 2). DNA molecules generated in a GEM share a common 10x Barcode. Libraries are generated and sequenced from the DNA molecules and 10x Barcodes are used to associate the individual reads back to the individual partitions.

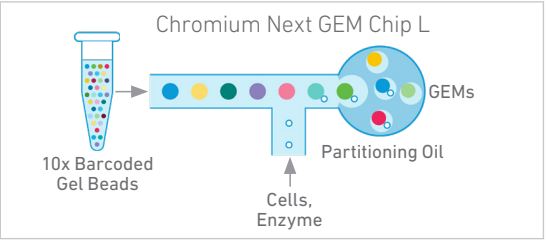


Figure 2. GEM generation in Chromium Next GEM Chip L .

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

Sample, Reagent & Workflow Comparison

		Single Cell 3' v3.1 (Standard)	Single Cell 3' LT v3.1 (Low Throughput)
Sample Prep	Sample specs.		
	Cells per sample	500-10,000 cells per sample	100-1,000 cells per sample
	Multiplet rate	~0.8% per 1,000 cells	~8.0% per 1,000 cells
	Cell loading conc.	700-1,200 cells/µl	100-600 cells/µl Refer to Cell Dilution Guidelines for more information
	Cell Recovery Rate	Up to 65%	Up to 35%
Library Prep	Reagent Kits		
	10x Genomics Reagents	16 & 4 rxn kits Chromium Next GEM Single Cell 3' Kit v3.1, 16 rxns PN-1000268, 4 rxns PN-1000269	4 rxn kits only Chromium Next GEM Single Cell 3' LT Kit v3.1, 4 rxns PN-1000325
		48 & 16 rxn kits Chromium Next GEM Chip G Single Cell Kit, 48 rxns PN-1000120, 16 rxns PN-1000127	16 rxn kits only (orderable as part of 1000325) Chromium Next GEM Chip L Single Cell Kit, 16 rxns PN-1000321 Refer to Product & Documents List for more information
	GEM Generation & Barcoding		
	Cell Suspension Volume Table	Volumes for 500-10,000 Targeted Cell Recovery using a cell stock conc. 700-1,200 cells/µl	Volumes for 100-1,000 Targeted Cell Recovery using a cell stock conc. 100-600 cells/µl
	Gel Bead	Single Cell 3' v3.1 Gel Beads	Single Cell 3' LT v3.1 Gel Beads
	Gel Bead Barcode Diversity	~3,500,000	~9,000 Refer to Multiplet Rate & Signal-to-noise sections to see impact of barcode diversity
	Chip Loading (Chips cannot be used interchangeably)		
		Chip G	Chip L Loading configuration & vols. unchanged
	Chromium Controller		
	Firmware Version	4.0 or higher	**4.0 or higher**
	Run time	~18 min	**~18 min**
Sequencing	Sequencing Type		
		Paired-end, dual indexing (single indexing reagent kits also available)	Paired-end, dual indexing only Sequencing depth & run parameters unchanged
Software	Data Analysis		
	Cell Ranger	Cell Ranger 3.0-6.0	Cell Ranger 6.0
	Assay chemistry display on Web Summary	Single Cell 3' v3	Single Cell 3' v3 LT

Cell Dilution Guidelines

The optimal cell loading concentration for the Single Cell 3' LT protocol is ~100-600 cells/µl. A single cell/nuclei suspension should be prepared as per guidelines provided in Demonstrated Protocols on the 10x Genomics Support site and diluted to the optimal concentration. The cell concentration and viability of the sample should be determined using a cell counting device, such as a hemocytometer or a Countess II FL Automated Cell Counter. Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells.

The key cell dilution guidelines for the LT assay are listed below and illustrated in Fig. 3.

- The optimal range of cell stock concentrations for the Single Cell 3' LT v3.1 assay is 100-600 cells/µl.
- Dilute cells using only compatible cell media/buffer (minimize carryover of media that can destabilize GEMs & impact GEM-RT reaction). Always mix cells gently and thoroughly before taking an aliquot.
- In instances where the initial cell count is already between 100-600 cells/µl, the cell stock may be used directly in the Master Mix for Single Cell 3' LT assay without further dilution. Note that cell count may not be accurate due to low concentration, which may impact cell recovery. Cell concentration below 100 cells/µl is not recommended for this assay.
- **One-step dilution:** If the initial cell stock concentration is 600-2,500 cells/µl, the cells may be diluted directly to 100-600 cells/µl.
- **Two-step dilution:** If the initial cell concentration is greater than 2,500 cells/µl, the cells may be diluted first to an optimal intermediate concentration of 700-2,500 cells/µl, recounted, followed by dilution to a final concentration 100-600 cells/µl.
- Once the cell concentration is adjusted to 100-600 cells/µl, refer to the Cell Suspension Volume Calculator Table in the relevant User Guides to prepare cells for Master Mix based on Targeted Cell Recovery.

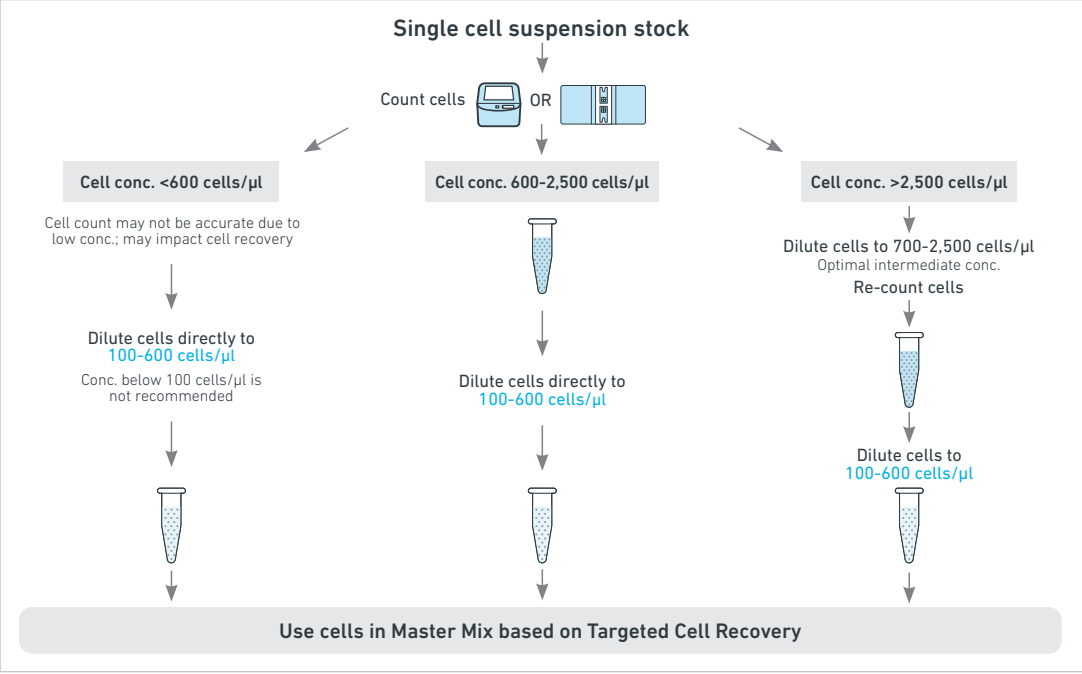


Figure 3. Cell dilution guidelines for Chromium Next GEM Single Cell 3' LT assay.

Multiplet Rate in Single Cell 3' LT v3.1 Assay

Multiplets are defined as two or more cells that have the same cell-associated barcode sequence. The multiplet rate in a single cell assay is dependent on the loading of cells in GEMs according to Poisson statistics and barcode collisions.

Poisson-derived multiplets:
During GEM generation, to achieve single cell resolution, cells are delivered at a limiting dilution following a Poisson distribution, such that the majority of generated GEMs contain no cell, the remainder largely contain a single cell (singlet), and a very small fraction of GEMs contain two or more cells (multiplets; Fig. 4A). Loading more cells into a channel increases the multiplet rate. For GEMs with singlets, the transcriptome of only one cell is indexed by a common 10x barcode. In contrast, multiplets in a GEM result in transcriptome from two or more cells being indexed by same barcode, preventing association of the transcriptome to the cell it was derived from.

Barcode collision-derived multiplets:
As a function of Gel Bead barcode diversity, different cells partitioned into physically distinct GEMs may still share the same barcode sequence, a phenomenon known as barcode collision (Fig. 4B). Although, the cells are not co-located in the same GEM, their transcriptome have the same 10x Barcode, making them indistinguishable from each other during data analysis.

In the standard Single Cell 3' v3.1 assay, the Gel Beads have high barcode diversity such that the number of Gel Bead barcodes significantly exceeds the number of partitions. Hence, the dominant source of multiplets in the standard assay are Poisson-derived (Fig. 4A). The barcode diversity of Single Cell 3' LT Gel Beads is ~10 times lower than the number of partitions available. Therefore, the dominant source of multiplets in the Single Cell 3' LT assay are derived from barcode collisions (Fig. 4B).

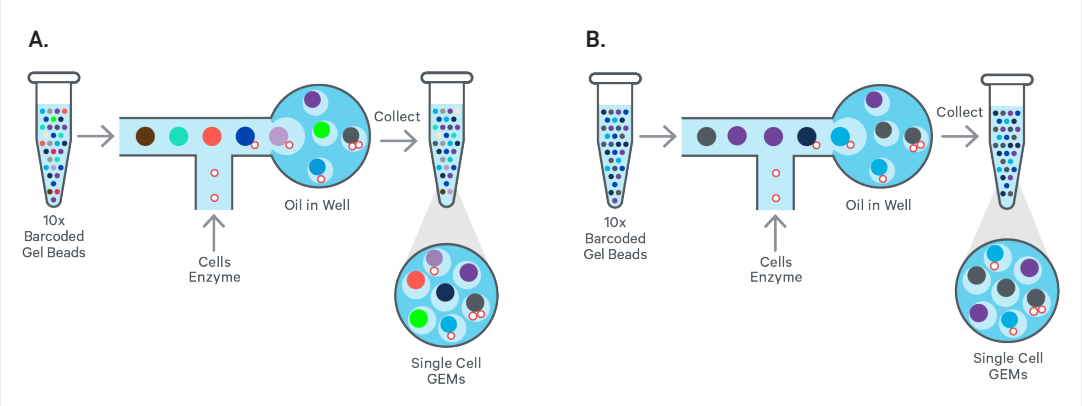


Figure 4. Schematic showing multiplets derived from Poisson distribution (A) and from both Poisson distribution and barcode collisions (B).

The table below shows empirically derived multiplet rate comparison for the standard Single Cell 3' v3.1 and the Single Cell 3' LT v3.1 assays.

Multiplet Rate (%)	# of Cells Recovered	
	Single Cell 3' v3.1 (Standard)	Single Cell 3' LT v3.1 (Low Throughput)
~0.8%	~1000	~100
~1.5%	~2000	~200
~2.3%	~3,000	~300
~3.0%	~4,000	~400
~3.8%	~5,000	~500
~4.6%	~6,000	~600
~5.3%	~7,000	~700
~6.1%	~8,000	~800
~6.8%	~9,000	~900
~8.0%	~10,000	~1,000

The multiplet rate data derived from human HEK293T and mouse NIH/3T3 cells that were mixed (1:1) and profiled using both the Single Cell 3' LT v3.1 and standard Single Cell 3' v3.1 assays is shown in Figure 5. The multiplet rate when normalized to the same cell load is ~10 fold higher for Single Cell 3' LT v3.1 (916 cells detected, 7.1% multiplets per 1,000 cells, Fig. 5A) compared to standard Single Cell 3' v3.1 (873 cells detected, 0.8% multiplets per 1,000 cells, Fig. 5B).

In summary, even though the multiplet rate for Single Cell 3' LT v3.1 is ~10-fold higher compared to Single Cell 3' v3.1, the absolute number of multiplets is kept consistent between the two assays. This done by only recovering up to 1,000 cells in the LT assay as opposed to recovering up to 10,000 cells in the standard assay. This has two important implications: Using the Single Cell 3' LT v3.1 assay to recover more than 1,000 cells will result in high multiplet rate. When targeting to recover only a few hundred cells, the multiplet rate for the LT assay will be ~10-fold higher than the standard Single Cell 3' v3.1 assay.

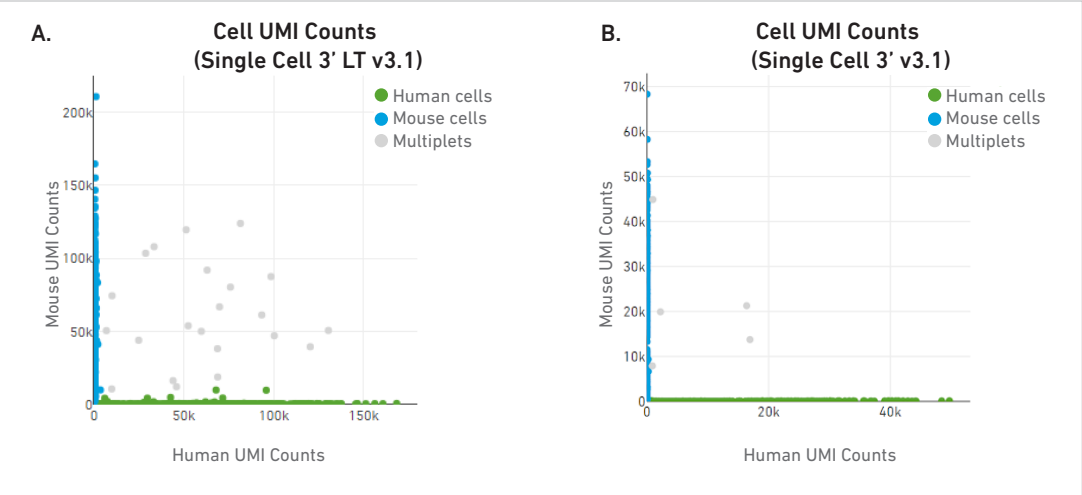


Figure 5. Scatter plot of human and mouse UMI counts detected in a mixture of HEK293T and NIH/3T3 cells. Cell barcodes mapping to human (green), mouse (blue) or both, multiplets (gray), are shown for (A) Single Cell 3' LT v3.1 assay (916 cells detected, 7.1% multiplets per 1,000 cells) compared to the standard Single Cell 3' v3.1 assay (873 cells detected, 0.8% multiplets per 1,000 cells).

Signal-to-noise Ratio

Gel Bead diversity impacts the signal-to-noise ratio in the data. The signal from cell-derived mRNA data is comparable between the Single Cell 3' LT v3.1 and standard Single Cell 3' v3.1 assay, while the background signal per 10x Barcode from ambient mRNA molecules is increased in the Single Cell 3' LT assay. This increase in background signal can be attributed to the lower LT Gel Bead barcode diversity, which increases barcode collision rate. As depicted in Fig 4B, barcode collisions result in significantly higher proportion of GEMs which contain Gel Beads that share the same barcode sequence. Hence, ambient mRNA present in physically distinct GEMs are tagged with the same barcode sequence, increasing the apparent noise level per-barcode.

The functional impact of this change in signal-to-noise profile is minimal, with the data quality remaining comparable between the LT and the standard assay. When profiling the same number of cells, the ambient mRNA background, or "knee" of the barcode rank plot is ~10-fold higher for Single Cell 3' LT data compared to the standard Single Cell 3' assay (Fig. 6A). However, when throughput is increased by running ~10-fold more cells for the standard Single Cell 3' v3.1 assay (8,428 cells) versus the low throughput Single Cell 3' LT v3.1 assay (892 cells), the barcode rank plots are comparable between the two datasets (Fig. 6B). This highlights that data derived using the low throughput Single Cell 3' LT v3.1 reagents can be extrapolated to gauge data quality of the sample when ~10-fold more cells are profiled using the standard Single Cell 3' v3.1 reagents.

The UMI density plots for cell surface protein CD11c assessed using the Feature Barcode technology in conjunction with the low throughput and standard assays show similar outcomes at the indicated cell loads (Figs. 6C-D).

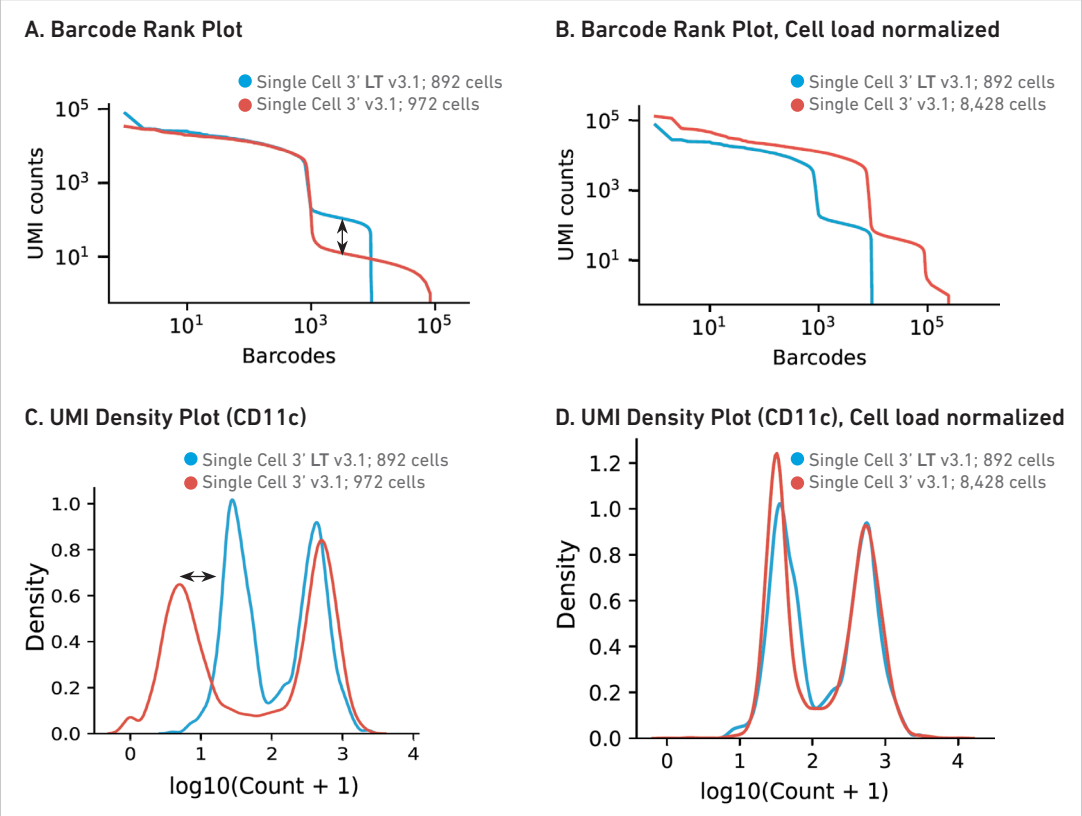


Figure 6. Barcode rank plots and antibody UMI density plots from data derived using the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays. The knee region of the barcode rank plot is ~10-fold higher for Single Cell 3' LT v3.1 data (892 cells) compared to the data from the standard Single Cell 3' v3.1 assay for a similar cell load (972 cells) (A). When ~10-fold more cells are used for the standard Single Cell 3' v3.1 assay, the barcode rank plots are comparable (B). Similar results are observed for CD11c UMI plots at the indicated cell loads for the two assay types (C, D).

Clustering Data at Low Cell Loads

Identification of distinct cell populations through clustering of single cell data is critical for gaining biological insights. Typically, sampling more cells increases cluster resolution through subdivision of key cell types and may thereby also enhance rare cell type detection.

Aggregated gene expression derived from Single Cell 3' LT v3.1 and Single Cell 3' v3.1 assays show comparable clustering along with similar relative abundance of major cell types (Fig. 10-12). The aggregated data were obtained by combining the hundreds of cells profiled using Single Cell 3' LT v3.1 with thousands of cells profiled using the standard Single Cell 3' v3.1 assay. Unsupervised clustering of the same gene expression data is shown in Figure 7. The t-SNE plots obtained from approximately the same number of cells profiled using the Single Cell 3' LT v3.1 (892 cells) and the Single Cell 3' v3.1 (927 cells) assays show similar clustering and comparable major cell type detection (Figs. 7A -7B). When the cell load was increased from hundreds to thousands of cells for the standard assay (8,428 cells; Fig. 7C), more clusters were identified from the same sample, namely Erythrocytes, Eosinophils, and Mast cells, alongside the other indicated lymphocyte subpopulations.

In summary, while low cell load data (Figs. 7A-7B) can provide valuable insights, for example, in a small-scale pilot study, assessing larger cell loads enables deeper insights with unbiased gene expression profiling in distinct cell types (Fig. 7C)). Therefore, the choice of cell throughput (low throughput versus the standard assay) should be based on specific research needs and desired experimental outcomes.

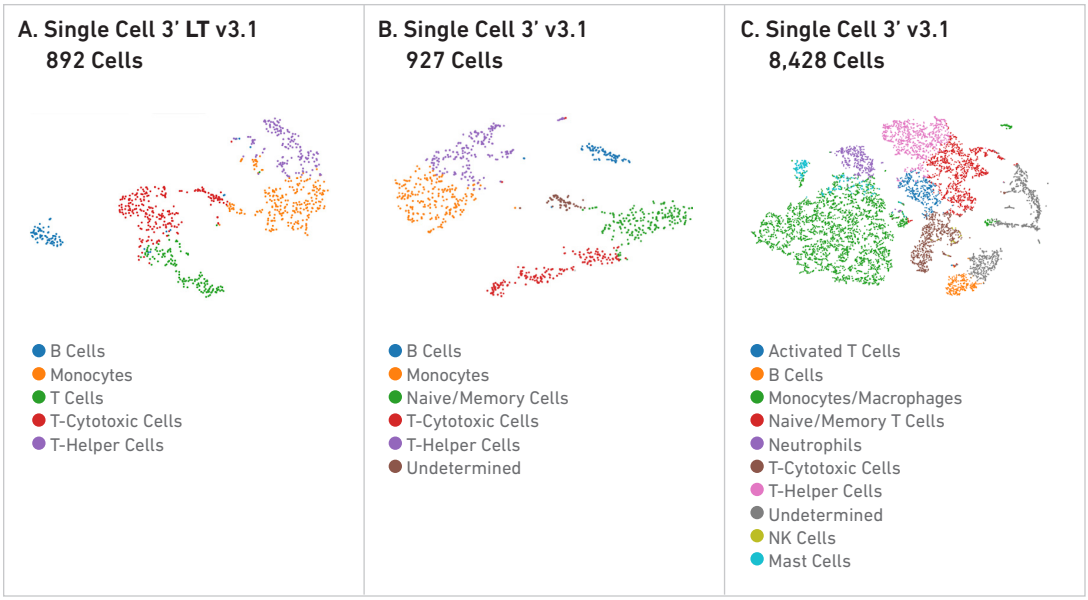


Figure 7. Unsupervised clustering and manual annotation of gene expression data derived from cells profiled using the Single Cell 3' LT v3.1 and Single Cell 3' v3.1 assays

Representative Data Highlights

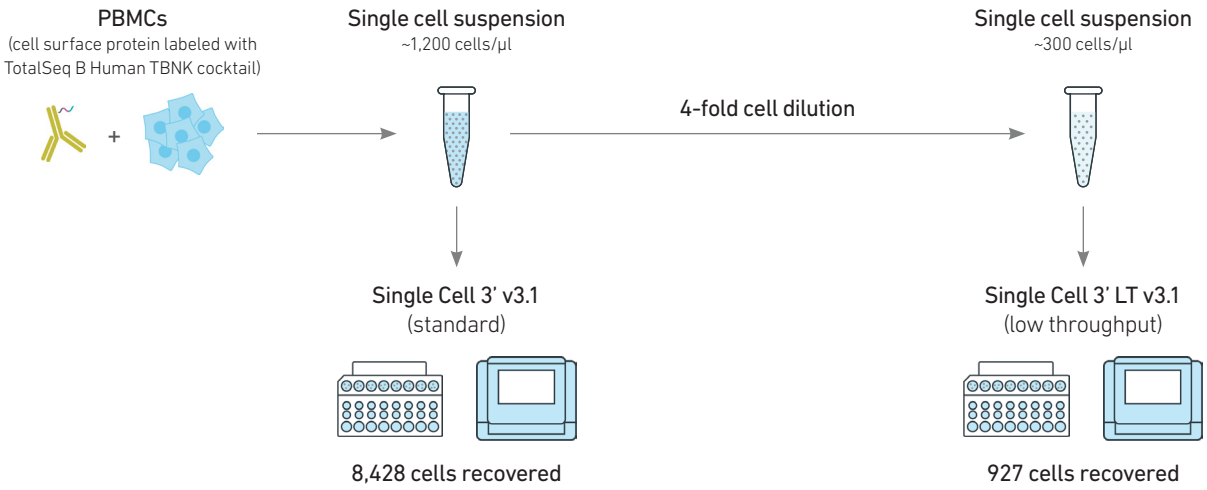
The Representative Data Highlights 1-2 provide a methods overview along with comparison of key results derived from various sample types. The libraries were generated using the specified Single Cell 3' LT v3.1 and standard Single Cell 3' v3.1 reagents and protocols, were sequenced, and the data were analyzed and visualized as indicated in each of the Data Highlights.

The results shown in Figures 8-15 clearly demonstrate that both low throughput and standard versions of Single Cell 3' v3.1 yield comparable data in terms of library complexity, mapping rates, gene expression, and cell surface protein detection.

Representative Data Highlight 1

Gene and Cell Surface Protein Expression

Methods Overview



	Gene Expression (GEX) + Cell Surface Protein Libraries	Gene Expression (GEX) + Cell Surface Protein Libraries
Protocol	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode-Cell Surface Protein (CG000317)	Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) with Feature Barcode-Cell Surface Protein (CG000400)
Sequencing	Depth: GEX-50,000, Protein-10,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90	Depth: GEX-50,000, Protein-10,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90
Analysis	Cell Ranger 6.0 Loupe Browser 5.0.1	Cell Ranger 6.0 Loupe Browser 5.0.1

Contd.

Results

Comparable library complexity and correlation

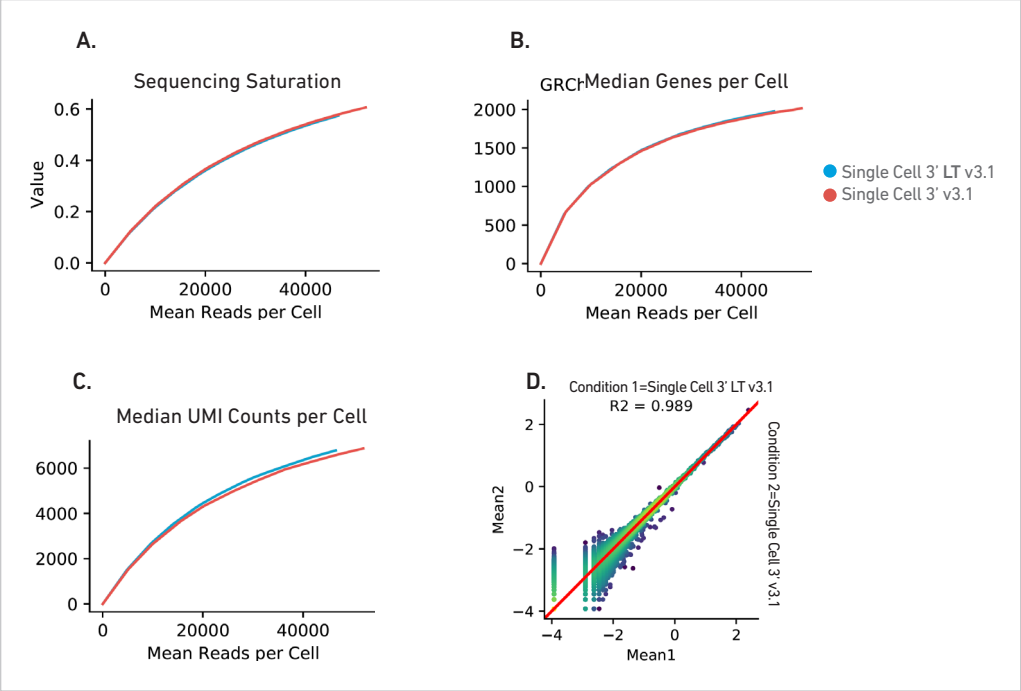


Figure 8. Comparable library complexity and chemistry correlation was observed between the Single Cell 3' LT v3.1 and Single Cell 3' v3.1 data. Comparable sequencing saturation (A), median genes per cell (B), median counts per cell (C), and the UMI correlation (D) plots were derived from the two assays.

Comparable read mapping rates

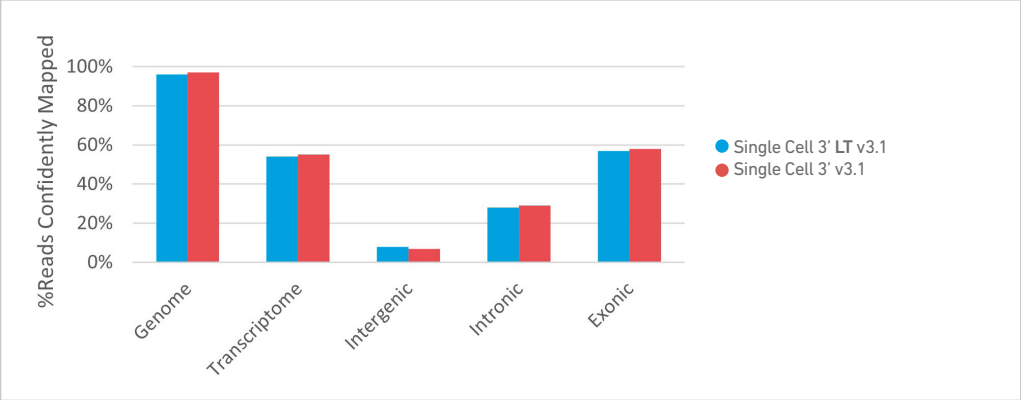


Figure 9. Comparable read mapping rates between the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 data.

Contd.

Results

Comparable gene expression based cell clustering data

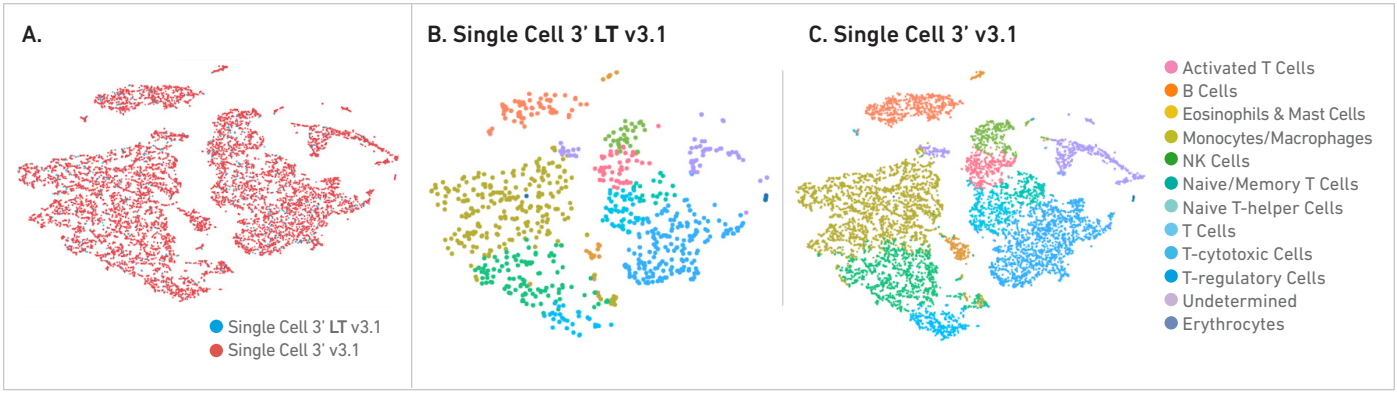


Figure 10. Similar cellular populations were detected in PBMCs profiled using aggregated gene expression data from the Single Cell 3' LT v3.1 (927 recovered cells) and the standard Single Cell 3' v3.1 (8,428 recovered cells) assays. Aggregated t-SNE plots from both assays (A), t-SNE plot from the low throughput assay (B), and t-SNE plot the standard assay (C) show comparable cell subpopulations based on gene expression data.

Comparable cell subpopulations based on gene expression data

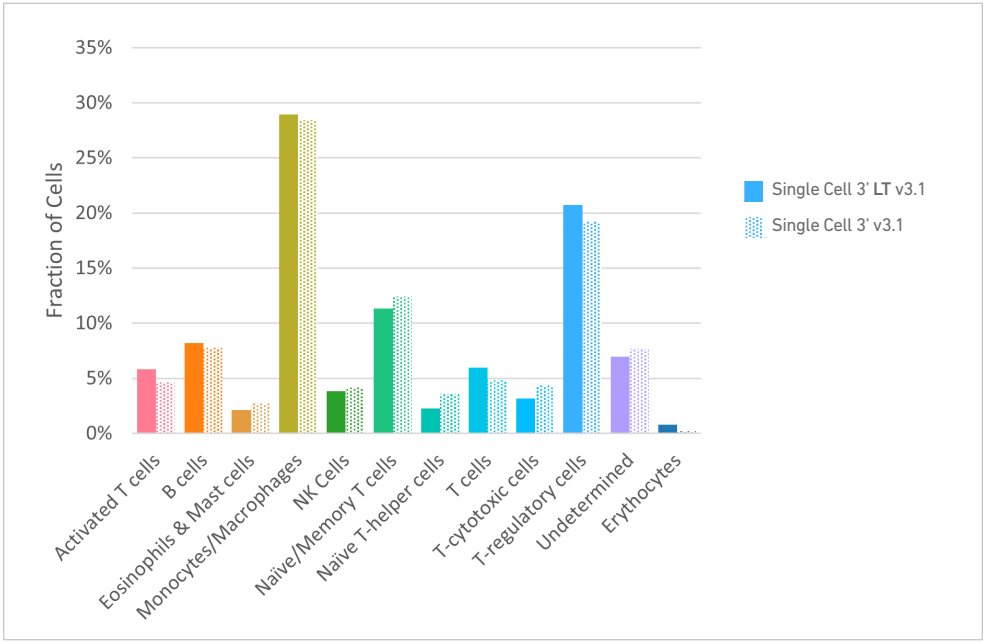


Figure 11. Similar percentages and relative proportions of known cellular populations were detected in gene expression data derived from Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays. Bar plot showing the relative abundance of cell subtypes identified.

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Results

Comparable cell surface protein based cell clustering and UMI counts

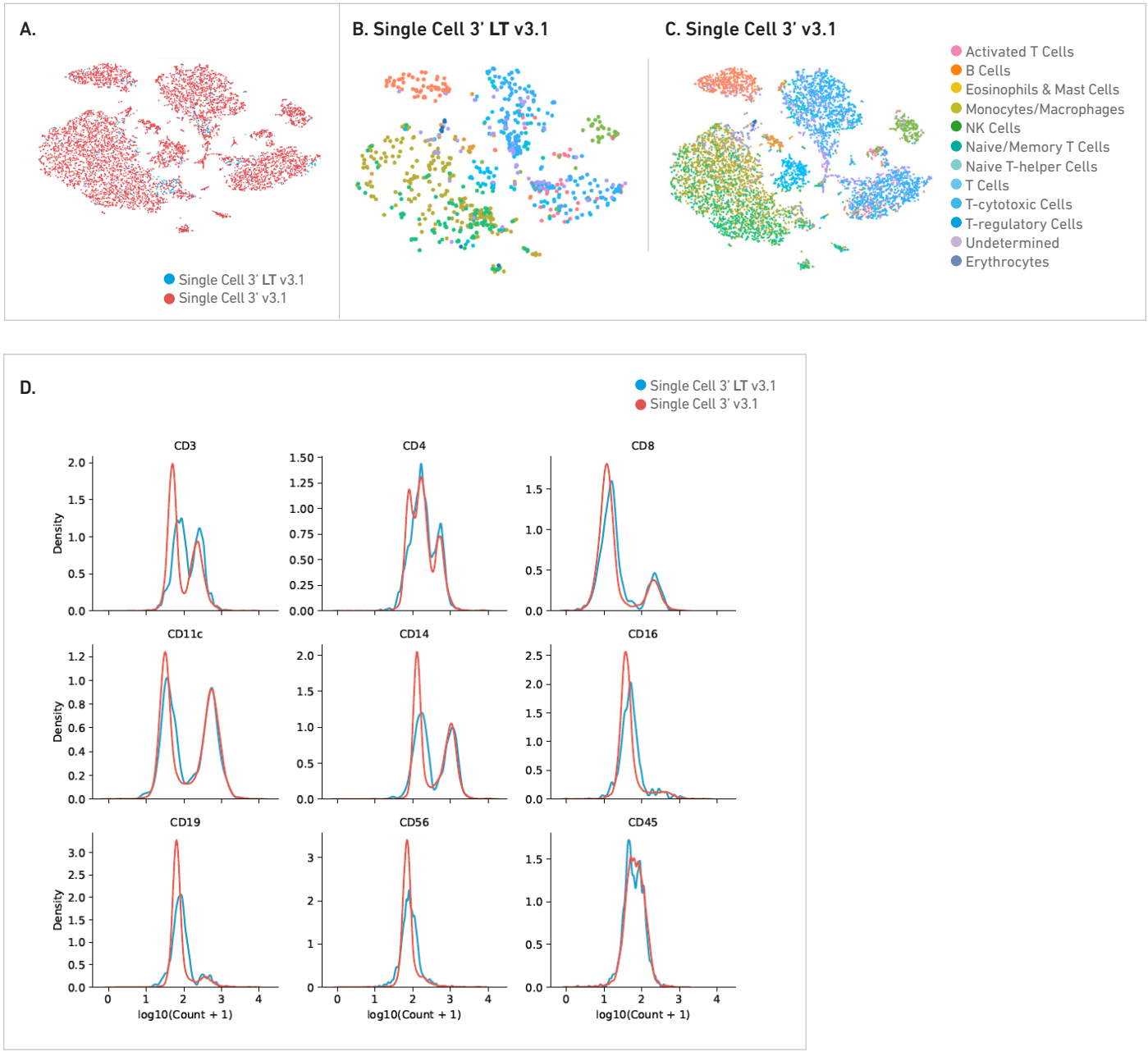
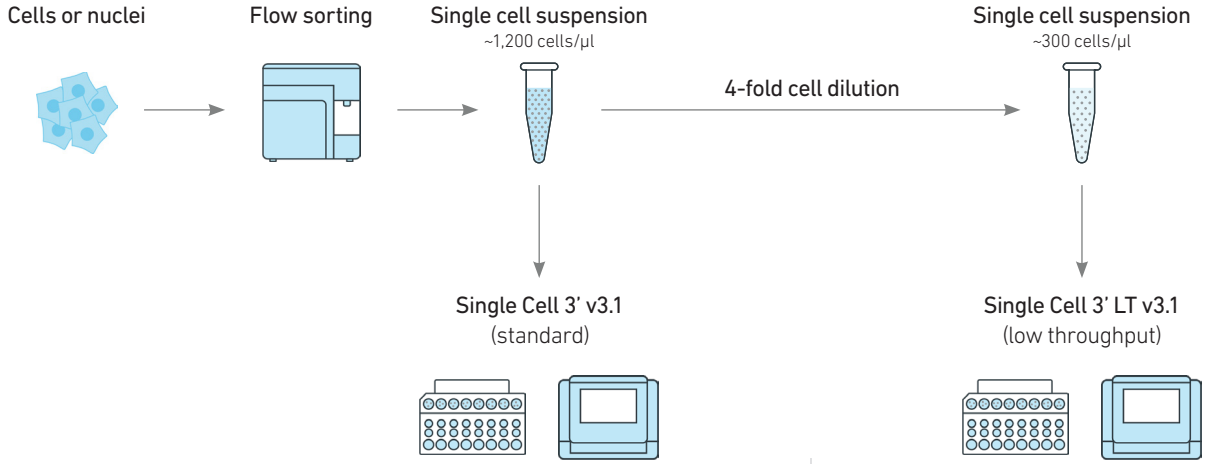


Figure 12. Similar cellular populations were detected using the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays. Aggregated t-SNE plots from both assays (A), t-SNE plot from the low throughput assay (B), and t-SNE plot the standard assay (C) show comparable cell subpopulations based on antibody based clustering data. UMI density plots (D) show comparable cell surface protein detection for the indicated proteins in data derived from the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays.

Representative Data Highlight 2

Gene Expression in Complex Sample Types

Methods Overview



Human cell types		
Brain	6,606 nuclei recovered	625 nuclei recovered
Invasive Ductal Carcinoma	5,676 cells recovered	688 cells recovered
Glioblastoma Multiforme	1,617 cells recovered	180 cells recovered
	Gene Expression (GEX) Libraries	Gene Expression (GEX) Libraries
Protocol	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) (CG000315)	Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) with Feature Barcode-Cell Surface Protein (CG000399)
Sequencing	Depth: GEX-60,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90	Depth: GEX-60,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90
Analysis Visualization	Cell Ranger 6.0 Loupe Browser 5.0.1	Cell Ranger 6.0 Loupe Browser 5.0.1

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Results

Comparable library complexity and correlation

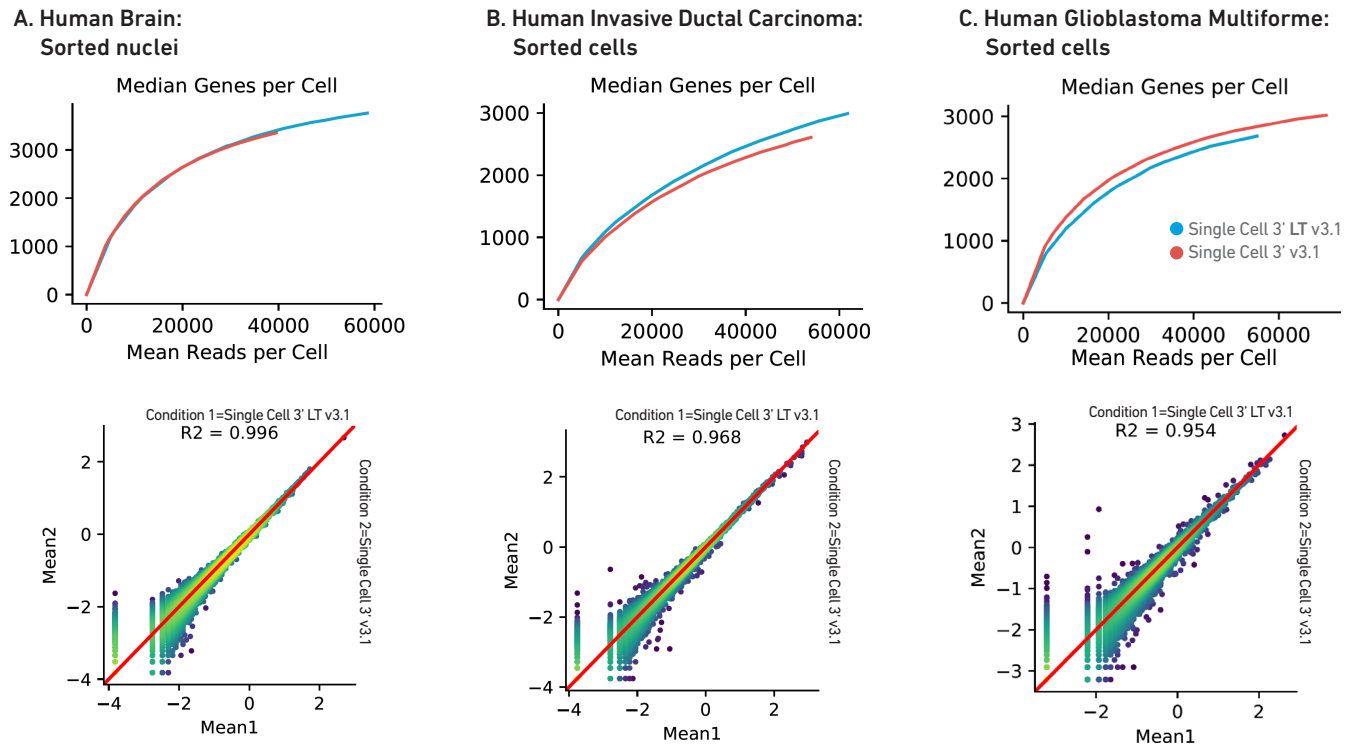

Figure 13. Comparable library complexity and correlation is observed in the gene expression data derived from Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays for three complex sample types. Comparable median genes per cell (top panels) and UMI correlation (bottom panels) plots derived from flow-sorted nuclei from Human Brain (A), flow-sorted cells from Human Invasive Ductal Carcinoma (B), and flow-sorted cells from Human Glioblastoma Multiforme (C).

Comparable mapping rates

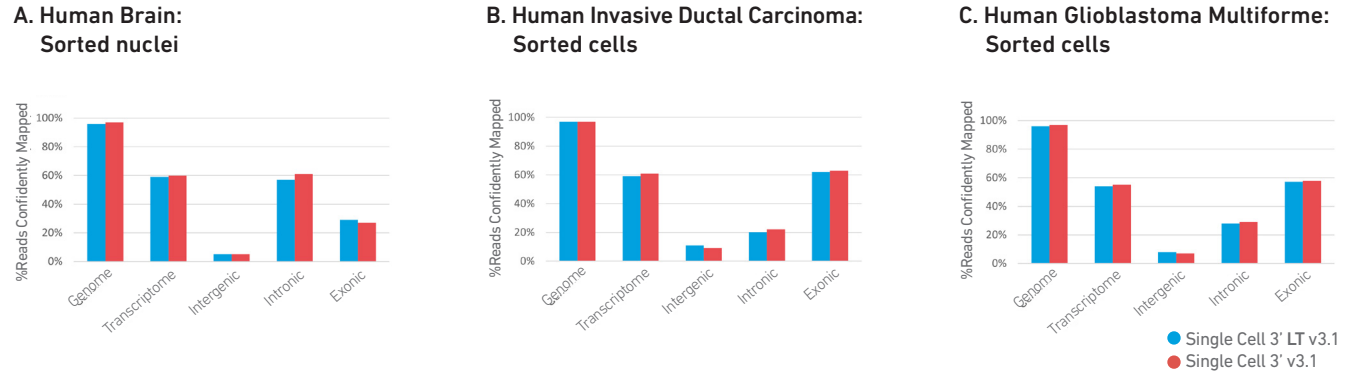

Figure 14. Comparable read mapping rates between the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 data for the indicated sample types.

Contd.

Representative Data Highlight 2 contd.

Results

Comparable cell clustering data

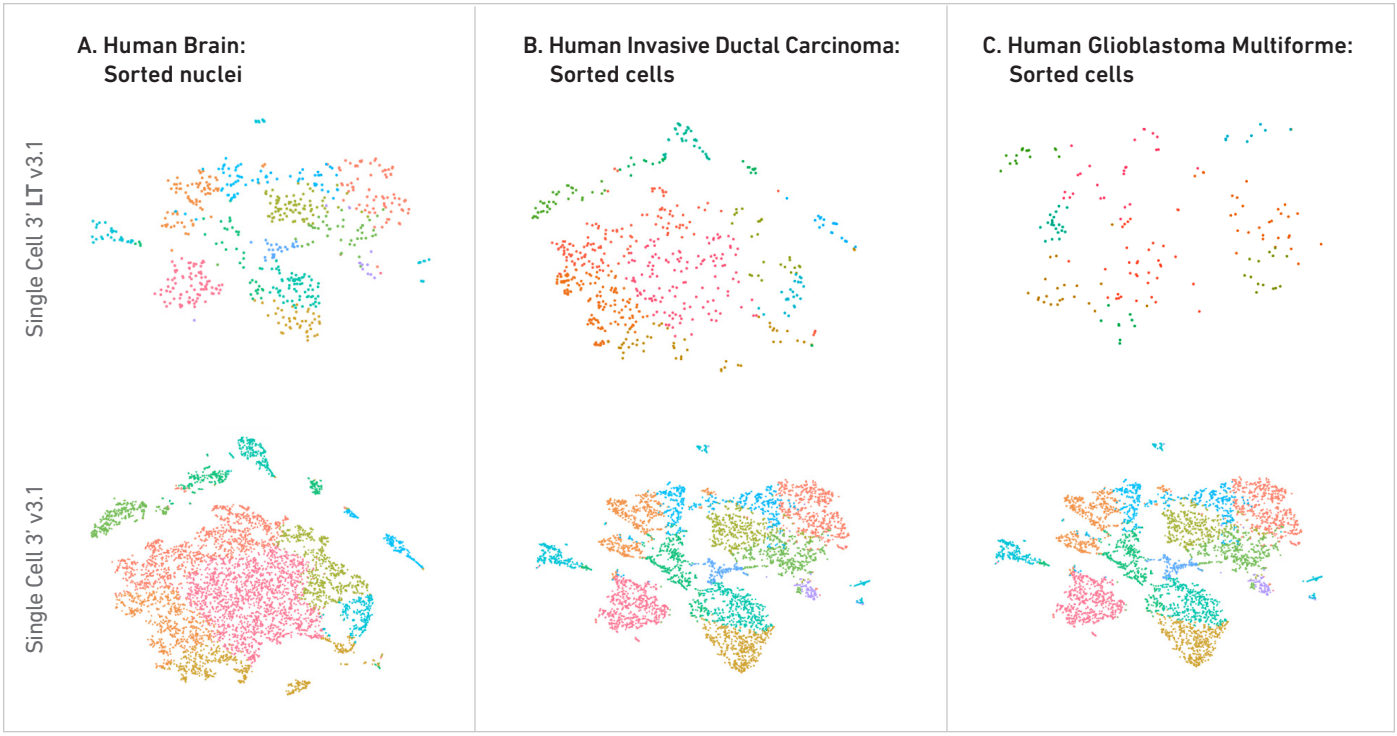

Figure 15. Similar cell clusters were detected in cells profiled using the Single Cell 3' LT v3.1 and the standard Single Cell 3' v3.1 assays. Comparable cell clusters between the data from low throughput assay (top panel) and the standard assay (bottom panels) in t-SNE plots derived from flow-sorted nuclei from Human Brain (A), flow-sorted cells from Human Invasive Ductal Carcinoma (B), and flow-sorted cells from Human Glioblastoma Multiforme (C).

Chromium Next GEM Single Cell 3' LT v3.1 – Product List & Documents

Product list for generating Chromium Single Cell 3' Gene Expression Libraries using the low throughput Single Cell 3' LT v3.1 assay :

REAGENT KITS	REACTIONS	PART NUMBER (PN)
Chromium Next GEM Single Cell 3' LT Kit v3.1	4 rxns	1000325
Chromium Next GEM Chip L Single Cell Kit	16 rxns	1000321 (orderable only as part of 1000325)
Dual Index Kit TT Set A	96 rxns	1000215
INSTRUMENT		
Chromium Controller & Next GEM Accessory Kit	-	120223 (12 month warranty) 120246 (24 month warranty)
SOFTWARE		
Cell Ranger Analysis Pipeline (DOWNLOAD)		
Loupe Cell Browser (DOWNLOAD)		
DOCUMENTS (for Single Cell 3' Gene Expression Libraries ONLY)		
User Guide : Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) (CG000399)		

If using Next GEM Single Cell 3' Reagent Kits LT v3.1 protocol with Feature Barcode technology, the 3' Feature Barcode Kit is required in addition to all the products listed above. Refer to the indicated documents for specific guidance.

REAGENT KITS	REACTIONS	PART NUMBER (PN)
3' Feature Barcode Kit	16 rxns	1000262
Dual Index Kit NT Set A	96 rxns	1000242
DOCUMENTS (for Single Cell 3' Gene Expression + Single Cell 3' Cell Surface Protein Libraries ONLY)		
User Guide : Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) with Feature Barcoding technology for Cell Surface Protein (CG000400)		

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA