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

Introduction

Chromium Next GEM Single Cell 3' HT v3.1 is a high throughput solution on the Chromium X series instrument for analyzing hundreds of thousands of cells per run, with up to 16 samples in a single chip. In combination with Feature Barcode technology, the high throughput assay also enables simultaneous cell surface protein detection, CRISPR screening, and cell multiplexing in single cells. This Technical Note highlights sample preparation, reagents, and workflow specifics for Single Cell 3' HT v3.1, along with information about data analysis. A comparison of representative data derived from the Single Cell 3' HT v3.1 assay versus the standard Single Cell 3' v3.1 assay is also presented.

Chromium Next GEM Single Cell 3' HT Workflow

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

Figure 1 provides a high level overview of the Single Cell 3' HT v3.1 workflow that includes a Chromium Next GEM chip designed to run up to 16 reactions on the Chromium X instrument.

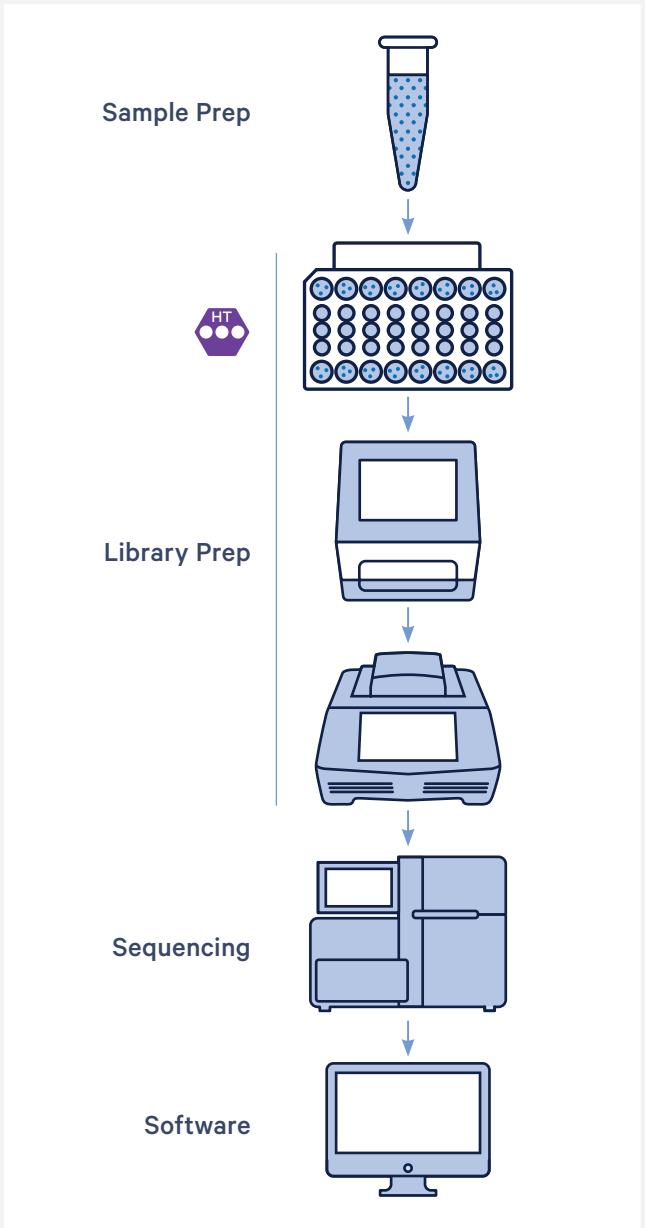


Figure 1. Chromium Next GEM Single Cell 3' HT v3.1 workflow.

The Chromium Next GEM Single Cell 3' HT v3.1 assay enables partitioning of 2,000–20,000 cells per channel of the Chromium Next GEM Chip M (2,000–60,000 cells per channel with CellPlex). Up to 16 samples loaded in two rows (labeled 2A & 2B) of the chip can be processed on the Chromium X per run. Nanoliter-scale Gel Beads-in-emulsion (GEMs) are generated by combining a Master Mix containing cells and enzymes, 10x Barcoded (~3,500,OOO barcodes) Single Cell 3' HT Gel Beads (loaded in row labeled 1), and Partitioning Oil (loaded in rows labeled 3A & 3B) onto the chip (Figure 2) and running the chip on Chromium X.

DNA molecules that are 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. Similar to the standard assay, Single Cell 3' Gene Expression libraries can be generated alone or in combination with Cell Surface Protein, CRISPR Screening, and Cell Multiplexing libraries using the high throughput assay.

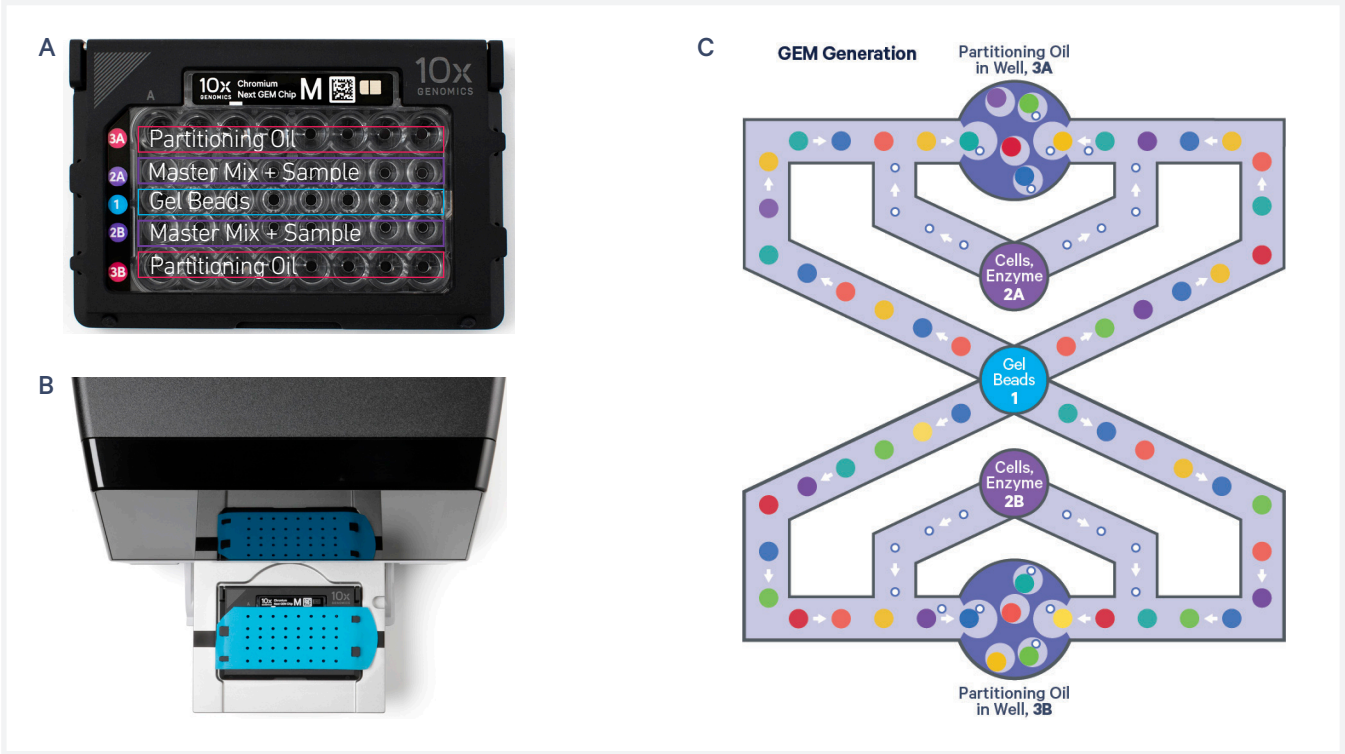
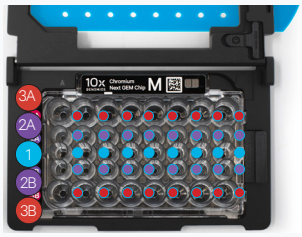
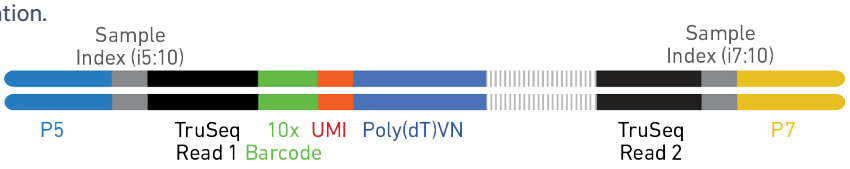


Figure 2. Chromium Next GEM Chip M is used for the Single Cell 3' v3.1 HT (high throughput) assay (A). Up to 16 samples loaded in two rows (labeled 2A & 2B) of the chip can be processed on the Chromium X per run (B). During the run (C), thousands of cells are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs) by combining a Master Mix containing cells and enzymes (rows labeled 2A & 2B) and 10x Barcoded Single Cell 3' HT Gel Beads (row labeled 1) in two microfluidic channels (for each sample) that connect with corresponding Partitioning Oil well (rows labeled 3A & 3B). GEMs are retrieved from rows 3A and 3B to generate sequencing-ready single cell libraries.

The key differences between the Single Cell 3' HT v3.1 and the standard Single Cell 3' v3.1 assays are presented in the table below. Refer to the relevant user guides for complete information.

	Single Cell 3' v3.1 *Standard*	**Single Cell 3' HT v3.1** *High throughput*
Sample Prep		
	Recommendations for preparing single cell suspensions are unchanged between the Single Cell 3' HT v3.1 and the standard Single Cell 3' v3.1 assays. Visit the 10x Genomics Support website for specifics Optimal cell stock concentration for chip loading is same for both assays. • 700-1,200 cells/µl • 1,300-1,600 cells/µl (with cell multiplexing)	
10x Genomics Reagents		
	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 16 rxn & 4 rxn kits	Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 48 rxn & 8 rxn kits *(See Product List & Documents for details)*
GEM Generation & Barcoding		
Master Mix Volume	31.9 µl/sample	**63.6 µl**/sample (no change in Master Mix reagents)
Cell Suspension Vol. Table	-	Updated volume
Gel Beads	Single Cell 3' Gel Bead v3.1	Single Cell 3' HT Gel Bead v3.1 *(in updated gel bead holder)*
Chip Loading		
	Chromium Next GEM Chip G is assembled in Next GEM Secondary Holder Chip Gasket attached *after* chip loading	Chromium Next GEM Chip M is assembled in Chromium X Secondary Holder Chip Gasket HT attached *before* chip loading
	Load up to 8 samples/chip	Load up to 16 samples/chip *Only an even number of reactions can be run on this chip*
		
	70 µl Master Mix + Sample - row labeled 1 50 µl Gel Beads - row labeled 2 45 µl Partitioning Oil - row labeled 3 *Bottom row is NO FILL*	**130 µl** Gel Beads - row labeled 1 **140 µl** Master Mix + Sample - rows labeled 2A & 2B **140 µl** Partitioning Oil - rows labeled 3A & 3B -

	Single Cell 3' v3.1 *Standard*		Single Cell 3' HT v3.1 *High throughput*	
Instrument				
	Chromium Controller		-	
	Now also compatible with Chromium X/iX		Chromium X	
	Run time ~18 min		Run time **~18 min**	
GEM Transfer				
	Transfer 100 µl GEMs from row labeled 3		Transfer **90 µl** GEMs from row 3A twice *(total 180 µl)* Transfer **90 µl** GEMs from row 3B twice *(total 180 µl)*	
	100 µl GEMs/sample		**180 µl** GEMs /sample	
	1 chip well » transfer GEMs to 1 tube		1 chip well » transfer GEMs to 2 tubes	
cDNA Amplification				
cDNA Amp. PCR Cycles	**Targeted Cell Recovery/Well**	**PCR Cycles**	**Targeted Cell Recovery/Well**	**PCR Cycles**
	<500 cells	13	<12,000 cells	12
	500–6,000 cells	12	>12,000 cells	11
	>6,000 cells	11		
cDNA Cleanup	After cDNA Cleanup, for each sample the cleanup product is present in 1 tube.		After cDNA Cleanup, for each sample, recombine the cleanup product present in 2 tubes to 1 tube.	
Library Construction				
Single Cell 3' Gene Expression Library	The Single Cell 3' dual index libraries generated using the standard and the HT assay have the same configuration.			
Sequencing				
	Paired-end, dual indexing		Paired-end, dual indexing	
	-		Same sequencing parameters	
	Single Cell 3' v3.1 is also available as a single index kit. Sequencing type for these libraries is paired-end, single indexing		*Not available as single index kit*	
Software				
	Sequencing data derived from libraries generated using either protocol can be analyzed and visualized using the latest versions of Cell Ranger and Loupe Browser available on the 10x Genomics Support website.			



Multiplet Rate in Single Cell 3' HT 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. The table below shows empirically derived multiplet rate comparison for the standard Single Cell 3' v3.1 and the Single Cell 3' HT v3.1 assays. 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' HT v3.1 and standard Single Cell 3' v3.1 assays is shown in Figure 3. The multiplet rate when normalized to the same cell load is approximately half for Single Cell 3' HT v3.1 (1,558 multiplets in 18,293 cells detected, ~0.4% multiplets per 1,000 cells, Fig. 3B) compared to standard Single Cell 3' v3.1 (931 multiplets in 9,513 cells detected, ~0.8% multiplets per 1,000 cells, Fig. 3C).

A

Multiplet Rate	# of Cells Recovered	
	Single Cell 3' HT v3.1 *High throughput*	Single Cell 3' v3.1 *Standard*
0.8%	2,000	1,000
1.6%	4,000	2,000
2.4%	6,000	3,000
3.2%	8,000	4,000
4.0%	10,000	5,000
4.8%	12,000	6,000
5.6%	14,000	7,000
6.4%	16,000	8,000
7.2%	18,000	9,000
8.0%	20,000	10,000

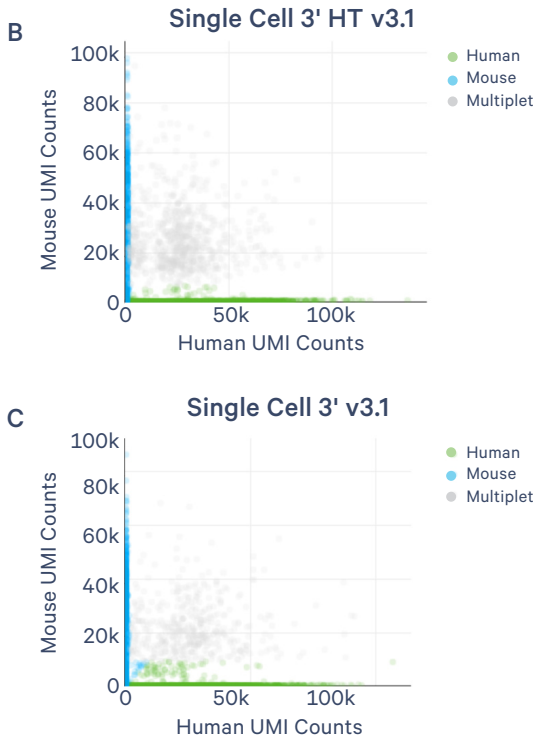


Figure 3. Multiplet rates based on cell recovery in Single Cell 3' HT v3.1 and standard assays (A). Scatter plot of human and mouse UMI counts detected in a mixture of HEK293T and NIH/3T3 cells. Cell barcodes mapping to human (green), mouse (blue) or both, multiplets (gray), are shown for (B) Single Cell 3' HT v3.1 assay (1,558 multiplets in 18,293 cells detected, 0.4% multiplets per 1,000 cells) compared to the standard Single Cell 3' v3.1 (C) assay (931 multiplets in 9,513 cells detected, 0.8% multiplets per 1,000 cells).

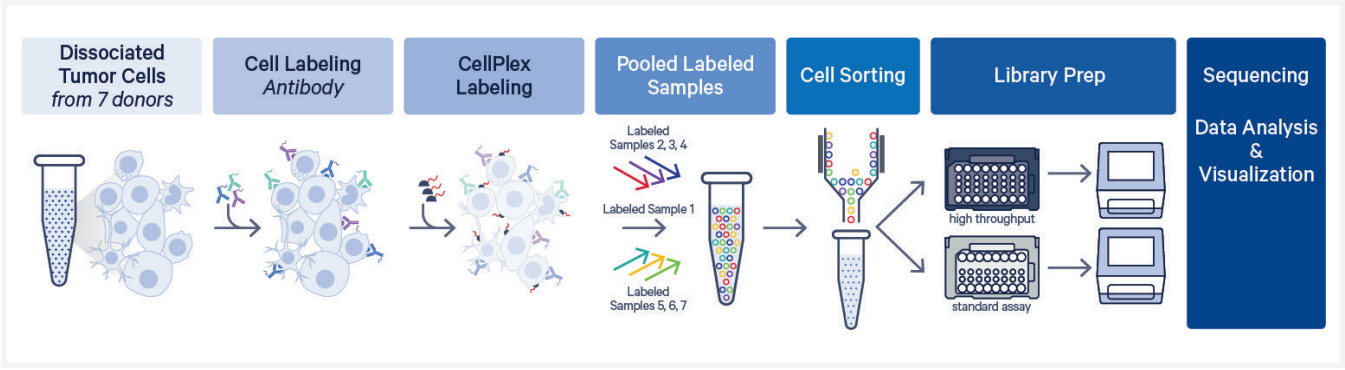
Results

The representative Data Highlight provides a Methods Overview along with comparison of key results derived from 7 non-small-cell lung cancer (NSCLC) patients. The libraries were generated using the specified Single Cell 3' HT v3.1 and standard Single Cell 3' v3.1 reagents and protocols, were sequenced, and the data were analyzed and visualized using Cell Ranger 6.1 and Loupe Browser.

The results shown in Figures 4-12 clearly demonstrate that the high throughput and standard Single Cell 3' v3.1 assays yield comparable data in terms of library complexity, mapping rates, gene expression, cell multiplexing, and cell surface protein detection. Additionally, the scale of the Single Cell 3' HT v3.1 assay further enhances the ability to detect rare cell types in these samples.

Data Highlight

Methods Overview



Dissociated tumor cells (DTCs) from 7 patients with non-small-cell lung cancer (NSCLC) were thawed and each sample was labeled with antibodies (TotalSeq B TBNK panel) for cell surface protein detection. Individual samples were then labeled with Cell Multiplexing Oligos (CMOs). All labeled samples were pooled, followed by cell sorting to remove dead cells (7-AAD+) and enrich for viable cells that were loaded separately onto a Single Cell 3' v3.1 Next GEM Chip M and Chip G targeting 40,000 and 20,000 cell recovery respectively. The chips were run on Chromium X followed by library preparation, sequencing, and data analysis as described in the respective user guides.

Comparable Library Complexity & Correlation

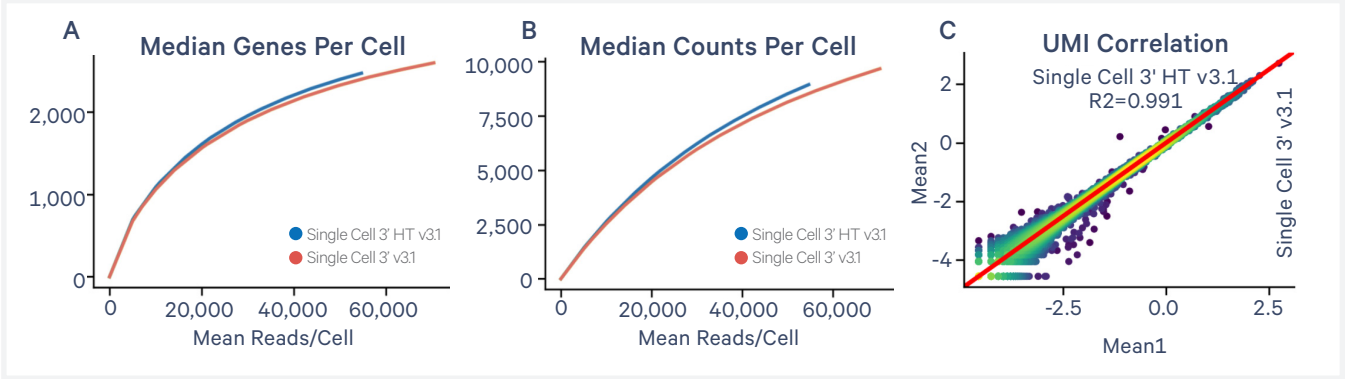


Figure 4. Comparable library complexity and chemistry correlation was observed between the data derived from Single Cell 3' HT v3.1 and Single Cell 3' v3.1 assays run on Chromium X. For the 36,293 cells recovered in the Single Cell 3' HT v3.1 assay and the 16,442 cells recovered in the standard assay, comparable median genes per cell (A), median counts per cell (B), and UMI correlation (C) were observed.

Data Highlight Contd.

Comparable Read Mapping Rates

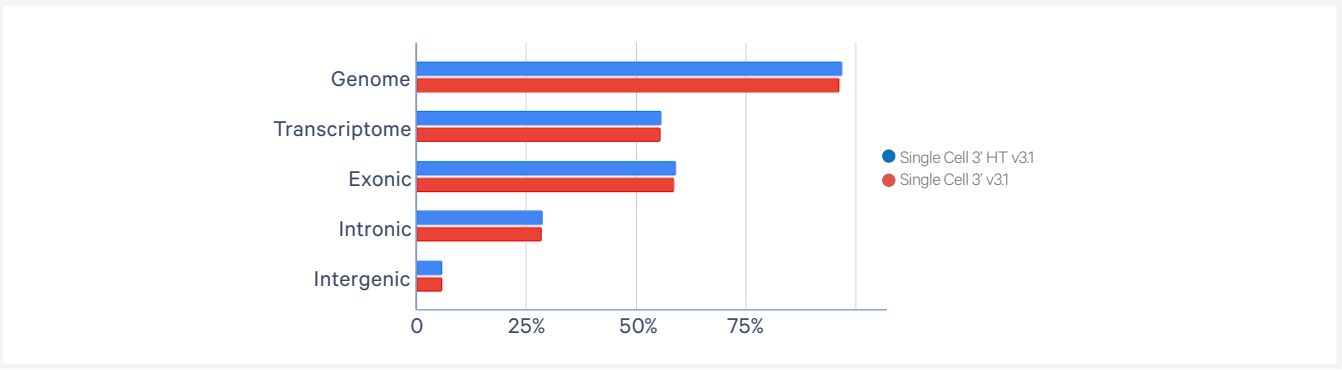


Figure 5. Comparable read mapping rates between the Single Cell 3' HT v3.1 and the standard Single Cell 3' v3.1 data.

Cell Multiplexing Oligo based Projection

Figure 6. Chromium Single Cell 3' Cell Multiplexing libraries derived from multiplexed dissociated tumor cell samples from each of the seven donors showed similar cell multiplex tag clustering in both the Single Cell 3' HT v3.1 (A) and standard Single Cell 3' v3.1 (B) data.

Overlapping Gene Expression Clustering & Cell Multiplexing Tag

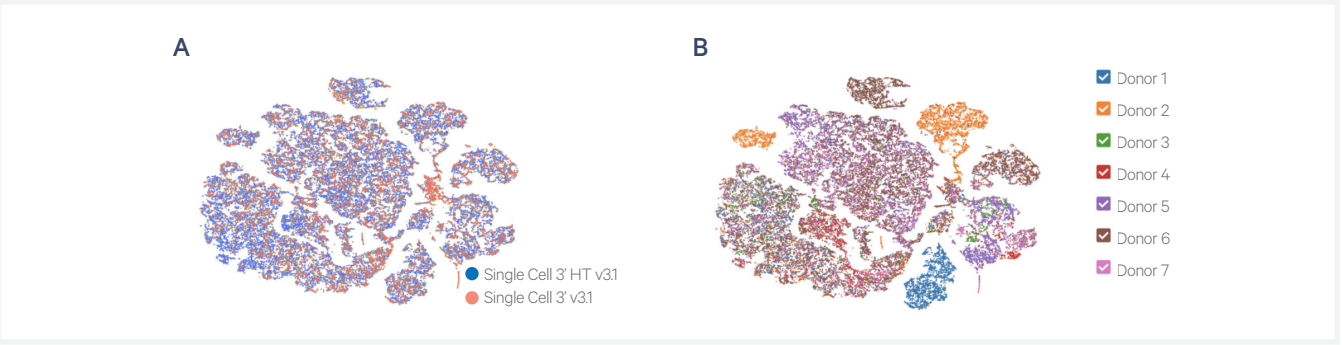


Figure 7. Overlapping Single Cell 3' Gene Expression clustering data derived from the Single Cell 3' HT v3.1 and standard Single Cell 3' v3.1 assays is shown in the aggregated t-SNE plot (A). The same plot with the superimposed data from 7 donors (based on cell multiplexing tags) is shown in panel B. Donor-specific clusters are observed, highlighting the diversity within 7 cancer patients.

Data Highlight Contd.

Gene Expression Based Cell Clustering & Cell Type Identification

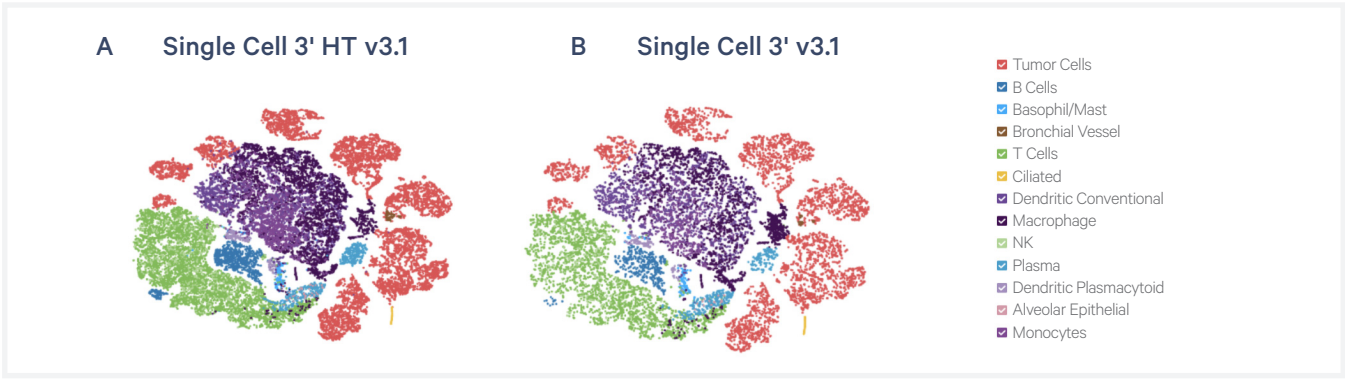


Figure 8. Similar cell clusters and cellular populations were detected in the tumor cells profiled using aggregated gene expression data from the Single Cell 3' HT v3.1 (A) and the standard Single Cell 3' v3.1 assays (B). The t-SNE plots show overlapping gene expression based cell clustering along with highly concordant cell type identification based on manual annotation.

Cell Surface Protein Based Cell Identification

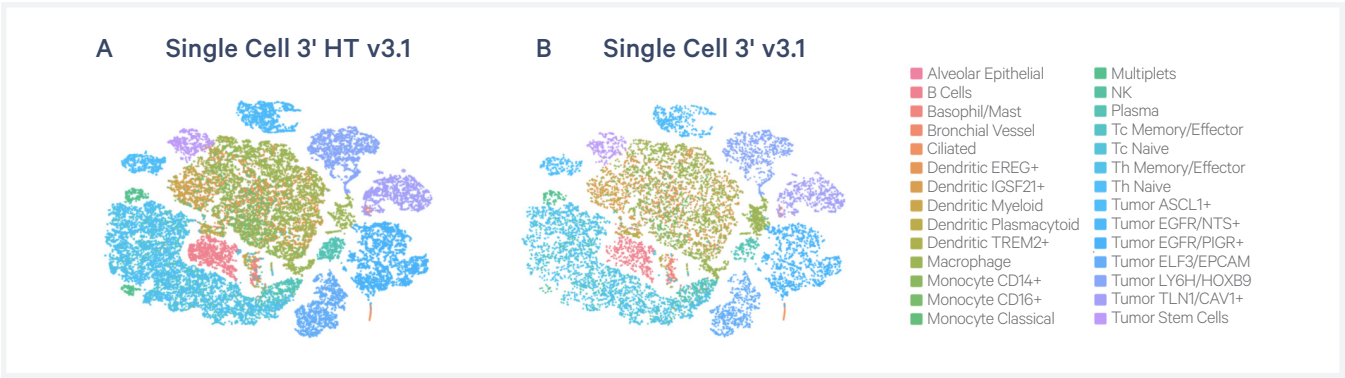


Figure 9. Chromium Single Cell 3' Gene Expression and Cell Surface Protein libraries generated using the Single Cell 3' HT v3.1 (A) and the standard Single Cell 3' v3.1 (B) assays show comparable cell type identification. The cell types were manually annotated based on single cell gene expression and data derived from cells labeled with TotalSeq B TBNK antibody panel.

Tumor Gene Expression Clusters

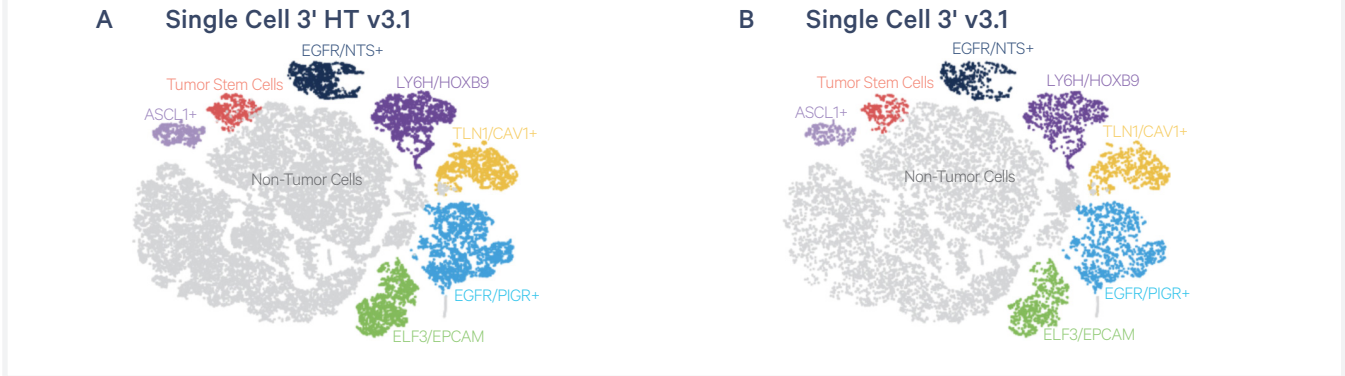


Figure 10. Seven comparable tumor gene clusters were observed in manually annotated data derived using the Single Cell 3' HT v3.1 (A) and the standard Single Cell 3' v3.1 (B) assays.

Data Highlight Contd.

Cell Type Identification

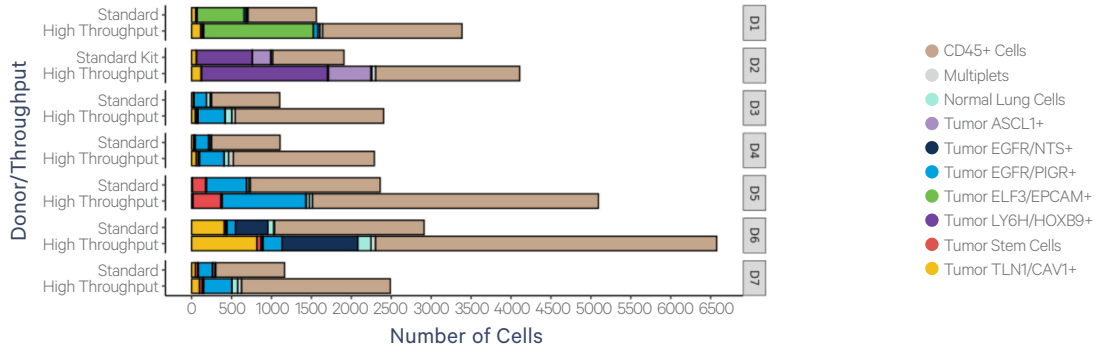


Figure 11. Tumor cells derived from 7 donors were manually annotated based on cell surface protein data derived using the Single Cell 3' HT v3.1 and the standard Single Cell 3' v3.1 assays. For each of the 7 donors (D1-D7), the graph shows absolute number of identified cell types for both assays, including CD45+ immune cells, normal lung cells, and various other tumor cells. Each donor displays a unique profile of tumor cell populations with specific genes upregulated. Additional rare cell types were detected in the Single Cell 3' HT v3.1 data

Rare Tumor Cell Type Detection

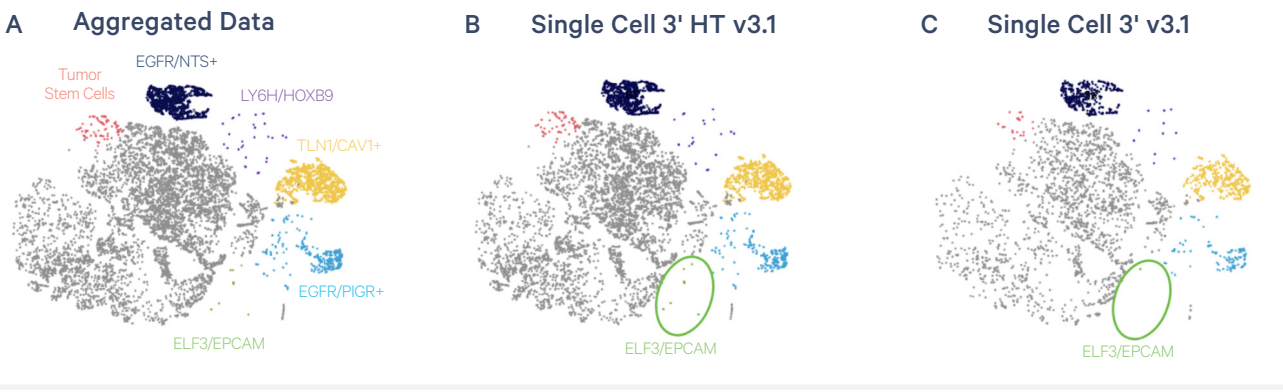


Figure 12. Data derived from Donor 6 sample shows overlapping Single Cell 3' Gene Expression for 6 out of 7 tumor clusters between the Single Cell 3' HT v3.1 and standard Single Cell 3' v3.1 assays as shown the aggregated t-SNE plot (A) The individual t-SNE plots derived from the two assays (B, C), show detection of the cluster (ELF3/EPCAM+) only in the Single Cell 3' HT v3.1 data. These results indicate that the scale of the Single Cell 3' HT v3.1 assay enables identification of rare tumor clusters to further enhance the understanding of tumor physiology.

Conclusions

The data generated using the Single Cell 3' HT v3.1 and the standard Single Cell 3' v3.1 assays are comparable in terms of library complexity, mapping rates, gene expression, and cell surface protein based cell detection. The ability to annotate cell types is further enhanced in Single Cell 3' HT v3.1 assay due to the ability to multiplex up to 60,000 cells per chip channel.

The Single Cell 3' HT v3.1 data also highlights different tumor markers present in samples from various donors along with detection of rare cell types. These can be powerful tools for understanding tumor physiology and enabling discovery of better drug targets.

Chromium Next GEM Single Cell 3’ HT v3.1 – Product List & Documents

Product list for generating Chromium Single Cell 3’ Gene Expression Libraries using the high throughput Single Cell 3’ HT v3.1 assay :		
Reagent Kits	**Reactions**	**Part Number (PN)**
Chromium Next GEM Single Cell 3' HT Kit v3.1	48 rxns 8 rxns	1000348 1000370
Chromium Next GEM Chip M Single Cell Kit	80 rxns 16 rxns	1000349 *(orderable only with 1000348)* 1000371 *(orderable only with 1000370)*
Dual Index Kit TT Set A	96 rxns	1000215
Additional kits for Feature Barcode technology protocols		
3’ Feature Barcode Kit	16 rxns	1000262
3' CellPlex Kit	48 rxns	1000261
Dual Index Kit NT Set A	96 rxns	1000242
Dual Index Kit NN Set A	96 rxns	1000243
Instrument		
Chromium X Upgrade Kit		1000331 *(12 month warranty)* 1000332 *(24 month warranty)*
User Guides		
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index)	CG00416	
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein	CG00417	
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening	CG00418	
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Multiplexing	CG00419	
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Cell Multiplexing	CG00420	
Chromium Next GEM Single Cell 3’ HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening & Cell Multiplexing	CG00421	
Chromium X Series (X/iX) with Readiness Test	CG00396	
Software		
Cell Ranger Analysis Pipeline (DOWNLOAD)		
Loupe Browser (DOWNLOAD)		

Document Revision Summary

Document Number	CG000422
Title	Chromium Next GEM Single Cell 3' HT v3.1: Reagents, Workflow & Data Overview
Revision	Rev A
Revision Date	August 2021

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