Chromium Next GEM Single Cell 5' HT v2: Reagents, Workflow & Data Overview

Introduction

Chromium Next GEM Single Cell 5' HT v2 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 in single cells. This Technical Note highlights sample preparation, reagents, and workflow specifics for Single Cell 5' HT v2, along with information about data analysis. A comparison of representative data derived from the Single Cell 5' HT v2 assay versus the standard Single Cell 5' v2 assay is also presented.

Chromium Next GEM Single Cell 5' HT Workflow

Chromium Next GEM Single Cell 5' HT v2 workflow (referred to as high throughput or HT) is similar to the Chromium Next GEM Single Cell 5' v2 workflow (referred to as standard), with specific updates that are indicated by an "HT" icon adjacent to the updated steps in the Single Cell 5' HT v2 User Guides (see Product List & Documents section for link to user guides).

Figure 1 provides a high level overview of the Single Cell 5' HT v2 workflow that includes a Chromium Next GEM chip designed to run up to 16 reactions on the Chromium X instrument.

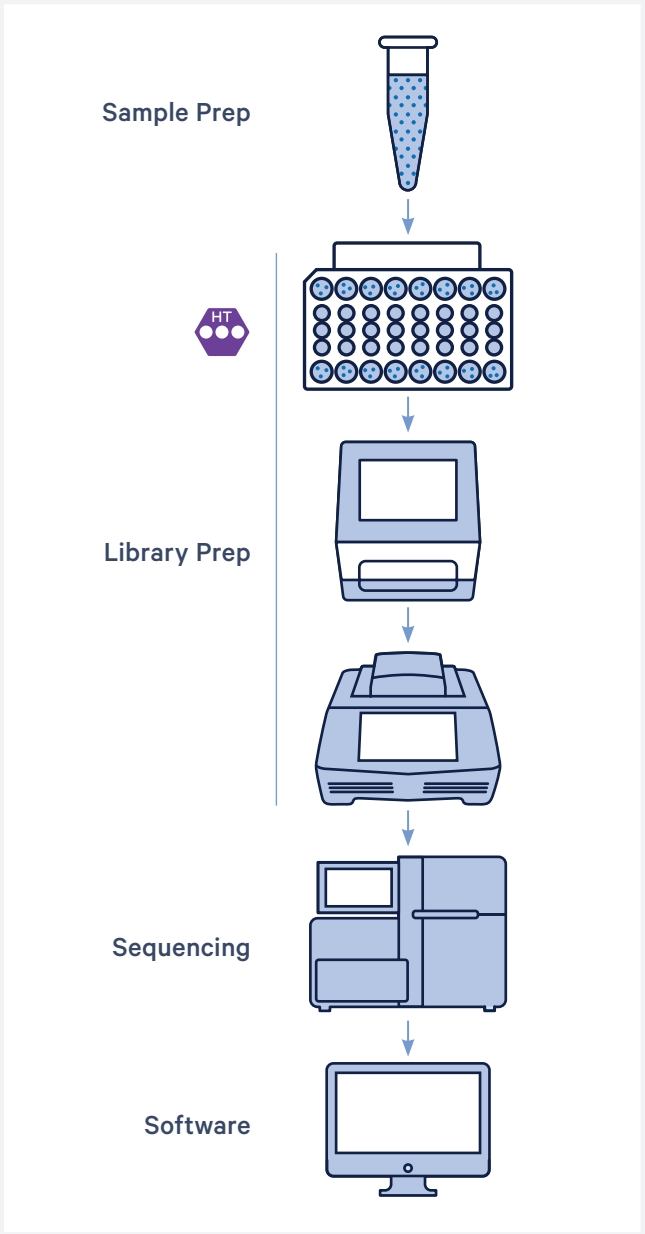


Figure 1. Chromium Next GEM Single Cell 5' HT v2 workflow.

The Chromium Next GEM Single Cell 5' HT v2 assay enables partitioning of 2,000–20,000 cells per channel of the Chromium Next GEM Chip N. 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 Single Cell 5' HT Gel Beads (loaded on row labeled 1), and Partitioning Oil (loaded on rows labeled 3A & 3B) onto the chip (Figure 2) and running the chip on Chromium X.

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. Similar to the standard assay, Single Cell 5' Gene Expression and V(D)J libraries can be generated alone or in combination with Cell Surface Protein libraries using the high throughput assay.

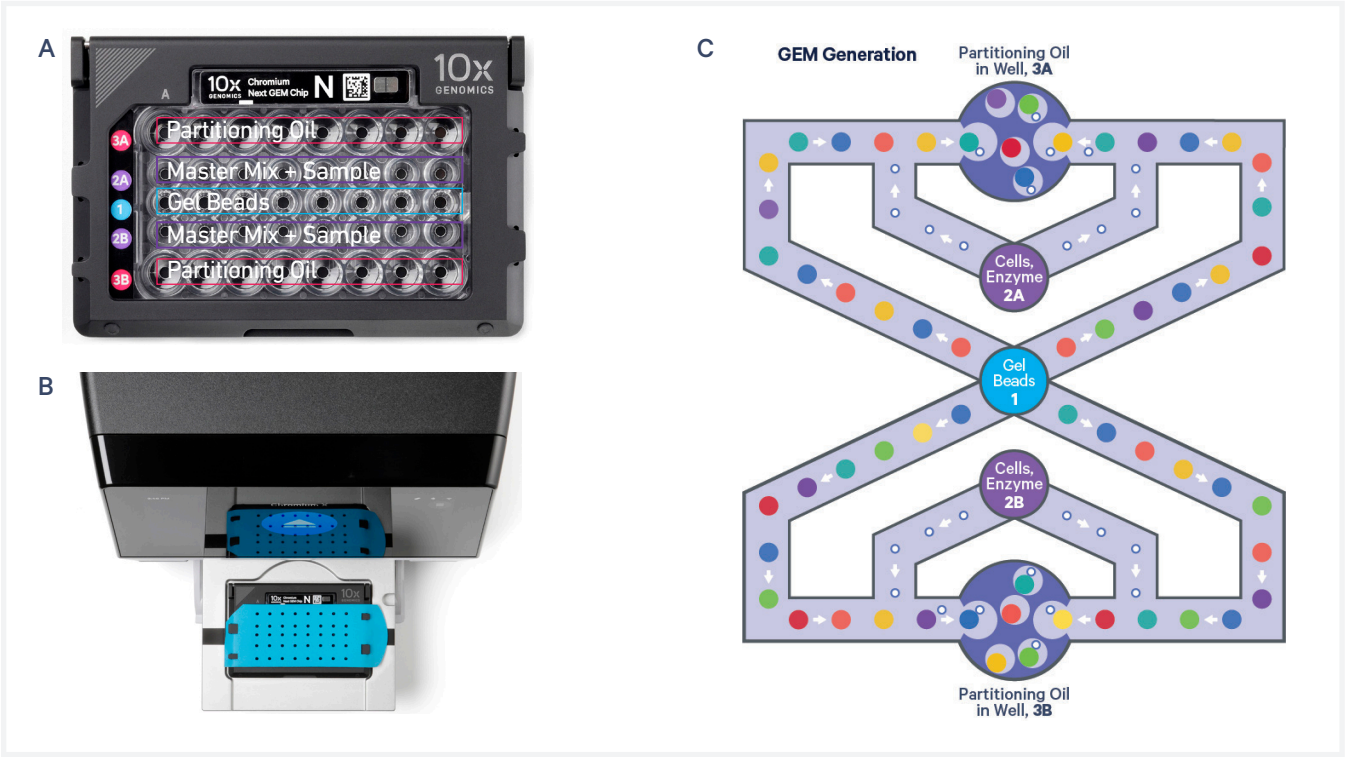
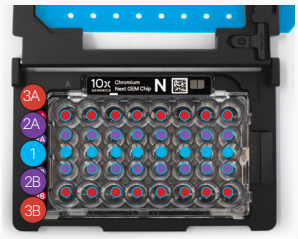


Figure 2. Chromium Next GEM Chip N is used for the Single Cell 5' v2 HT (high throughput) assay (A). Up to 16 samples loaded in two rows (labeled 2A & 2B) of the chip can be processed on the Chromium X per run (B). During the run (C), thousands of cells are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs) by combining a Master Mix containing cells and enzymes (rows labeled 2A & 2B) and 10x Barcoded Single Cell 5' 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 5' HT v2 and the standard Single Cell 5' v2 assay are presented in the following section.

	Single Cell 5' v2 *Standard*	Single Cell 5' HT v2 *High throughput*
Sample Prep		
	Recommendations for preparing single cell suspensions are unchanged between the Single Cell 5' HT v2 and the standard Single Cell 5' v2 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	
10x Genomics Reagents		
	Chromium Next GEM Single Cell 5' Reagent Kits v2	Chromium Next GEM Single Cell 5' HT Reagent Kits v2
	16 rxn & 4 rxn kits	48 rxn & 8 rxn kits *(See Product List & Documents for details)*
GEM Generation & Barcoding		
Master Mix Volume	36.3 µl/sample	**72.6 µl**/sample (no change in Master Mix reagents)
Cell Suspension Vol. Table	-	Updated volume
Gel Beads	Single Cell 5' Gel Beads v2	Single Cell 5' HT Gel Bead v2 *(in updated gel bead holder)*
Chip Loading		
	Chromium Next GEM Chip K is assembled in Next GEM Secondary Holder Chip Gasket attached after chip loading	Chromium Next GEM Chip N 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 5' v2 *Standard*			Single Cell 5' HT v2 *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**	**Total Cycles Low RNA Cells**	**Total Cycles High RNA Cells**	**Targeted Cell Recovery**	**Total Cycles Low RNA Cells**	**Total Cycles High RNA Cells**
	500-2,000	16	14	≤4,000	16	14
	2,001-6,000	14	12	4,000-12,000	14	12
	6,001-10,000	13	11	>12,000	13	11
cDNA Cleanup	After cDNA Cleanup, for each sample the cleanup product is present 1 tube.			After cDNA Cleanup, for each sample recombine the cleanup product present in 2 tubes to 1 tube.		
Library Construction						
cDNA Carry Forward	Carry forward 50 ng amplified cDNA for 5' GEX Library Construction			Carry forward only **20 µl** Sample for 5' GEX Library Construction		
Recommended SI PCR Cycles	**cDNA Input (ng)**	**Total Cycles**		**cDNA Input (ng)**	**Total Cycles**	
	1-25	16		0.25-25	14-16	
	26-50	14		25-150	12-14	
				150-500	10-12	
				500-1,000	8-10	
				1,000-1,500	6-8	
				>1,500	5	
Single Cell 5' GEX Library	The Single Cell 5' Gene Expression dual index libraries generated using the standard and the HT assay have the same configuration.					

<table>
<tr><th></th><th>Single Cell 5’ v2
Standard</th><th>Single Cell 5’ HT v2
High throughput</th></tr>
<tr><td colspan="3">V(D)J Amplification and Library Construction</td></tr>
<tr><td>V(D)J Amplified Product Carry Forward</td><td>Carry forward 50 ng V(D)J amplified sample for V(D)J Library Construction</td><td>Carry forward only 10 µl V(D)J amplified sample for V(D)J Library Construction</td></tr>
<tr><td>Sample Index PCR Cycles</td><td>Total 8 cycles</td><td>Cycle number depends upon the 10 µl carry forward V(D)J amplified product concentration
<table><tr><th>V(D)J Amplified Product Input</th><th>Total Cycles</th></tr><tr><td><25</td><td>9</td></tr><tr><td>25-150</td><td>8</td></tr><tr><td>151-500</td><td>7</td></tr><tr><td>501-1,000</td><td>6</td></tr><tr><td>>1,000</td><td>5</td></tr></table></td></tr>
<tr><td>V(D)J Library</td><td colspan="2"> 
</td></tr>
<tr><td colspan="3">Sequencing</td></tr>
<tr><td></td><td>Paired-end, dual indexing</td><td>Paired-end, dual indexing</td></tr>
<tr><td></td><td>Same sequencing parameters</td><td>Same sequencing parameters</td></tr>
<tr><td colspan="3">Software</td></tr>
<tr><td></td><td colspan="2">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.</td></tr>
</table>

Multiplet Rate in Single Cell 5' HT v2 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 5' v2 and the Single Cell 5' HT v2 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 5' HT v2 and standard Single Cell 5' v2 assays is shown in Figure 3.

The multiplet rate when normalized to the same cell load is approximately half for Single Cell 5' HT v2 (1,598 multiplets in 19,632 cells detected, 0.4% multiplets per 1,000 cells, Fig. 3B) compared to standard Single Cell 5' v2 (861 multiplets in 10,140 cells detected, 0.8% multiplets per 1,000 cells, Fig. 3C).

A

Multiplet Rate	# of Cells Recovered	
	Single Cell 5' HT v2 *High throughput*	Single Cell 5' v2 *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

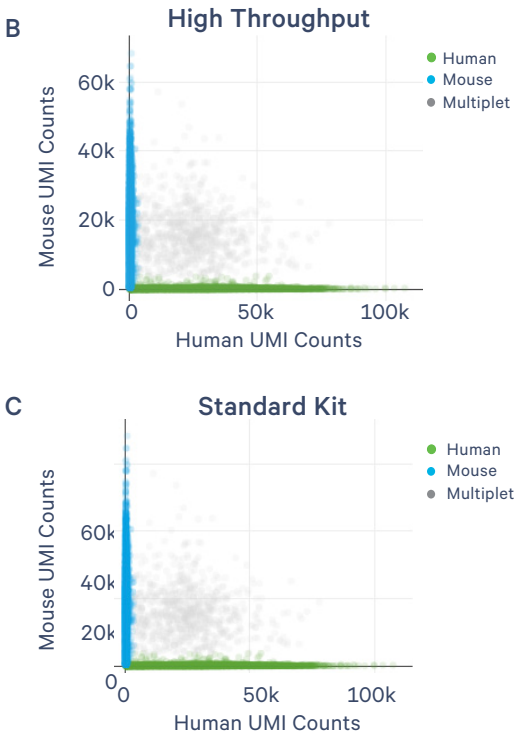


Figure 3. Multiplet rates based on cell recovery in Single Cell 5' HT v2 and standard assays (A). Scatter plot of human and mouse UMI counts detected in a mixture of HEK293T and NIH/3T3 cells. Cell barcodes mapping to human (green), mouse (blue) or both, multiplets (gray), are shown for (B) Single Cell 5' HT v2 assay (1,598 multiplets in 19,632 cells detected, 0.4% multiplets per 1,000 cells) compared to the standard Single Cell 5' v2 (C) assay (861 multiplets in 10,140 cells detected, 0.8% multiplets per 1,000 cells).

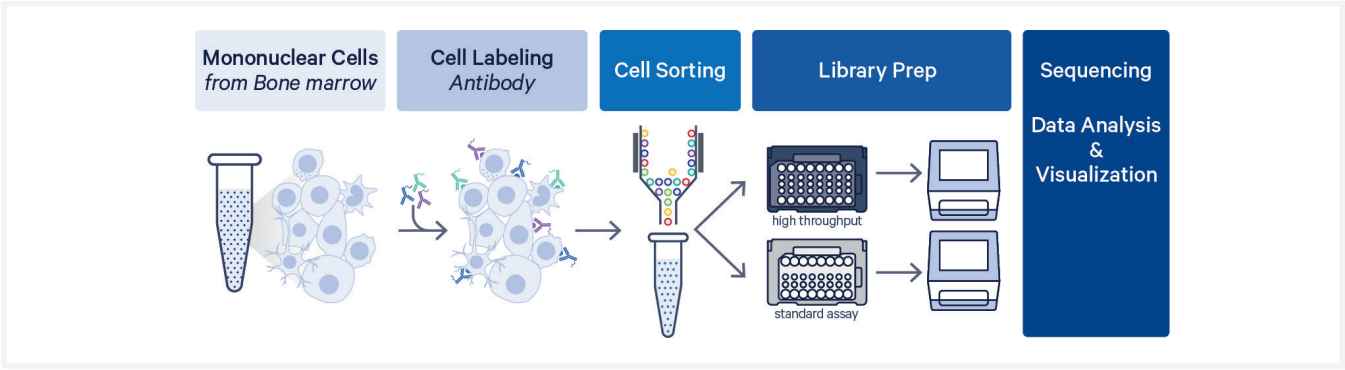
Results

The representative Data Highlight provides a methods overview along with comparison of key results derived from bone marrow mononuclear cells. The libraries generated using the specified Single Cell 5' HT v2 and standard Single Cell 5' v2 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 4-10 clearly demonstrate that the high throughput and standard Single Cell 5' v2 assays yield comparable data in terms of library complexity, mapping rates, gene expression, and cell surface protein detection. Additionally, the scale of the Single Cell 5' HT v2 assay further enables rare V(D)J clonotypes to be identified in these samples.

Data Highlight

Methods Overview



Mononuclear cells were isolated from fresh bone marrow aspirates following the Demonstrated Protocol Isolation of Leukocytes, Bone Marrow and Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing (CG000392) and cryopreserved. Thawed cells were labeled with antibodies (TotalSeq C Human Universal Cocktail) for cell surface protein detection. The labeled cells were sorted to remove dead cells (7AAD+) and enrich for viable cells that were loaded separately onto a Single Cell 5' v2 Next GEM Chip N (20,000 cells) and Chip K (10,000 cells) and were processed on Chromium X followed by library preparation, sequencing, and data analysis.

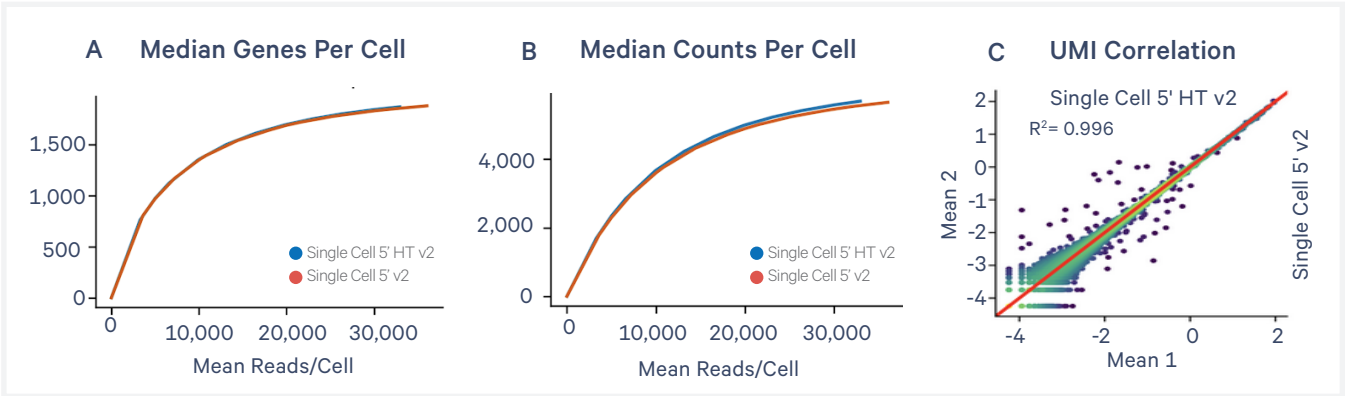


Figure 4. Comparable library complexity and chemistry correlation was observed between the data derived from Single Cell 5' HT v2 and Single Cell 5' v2 assays run on Chromium X. Comparable median genes per cell (A), median counts per cell (B), and the UMI correlation (C) plots are shown.

Data Highlight Contd.

Comparable Read Mapping Rates

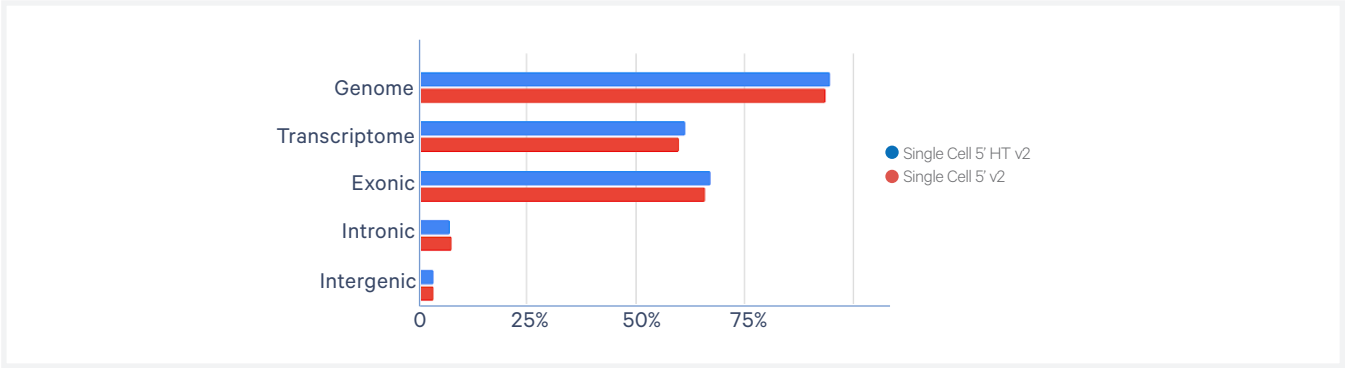


Figure 5. Comparable read mapping rates between the Single Cell 5' HT v2 and the standard Single Cell 5' v2 data.

Gene Expression Clustering

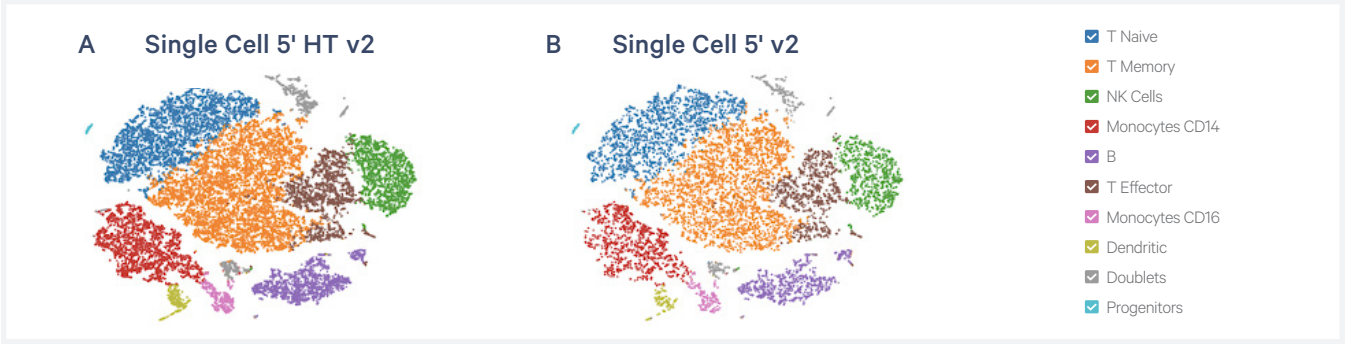


Figure 6. Single Cell 5' Gene Expression clustering data derived from the Single Cell 5' HT v2 (A) and standard Single Cell 5' v2 assays (B) is shown. Comparable t-SNE clusters and cell types were observed between the Single Cell 5' HT v2 and the standard Single Cell 5' v2 data.

Cell Type Annotation

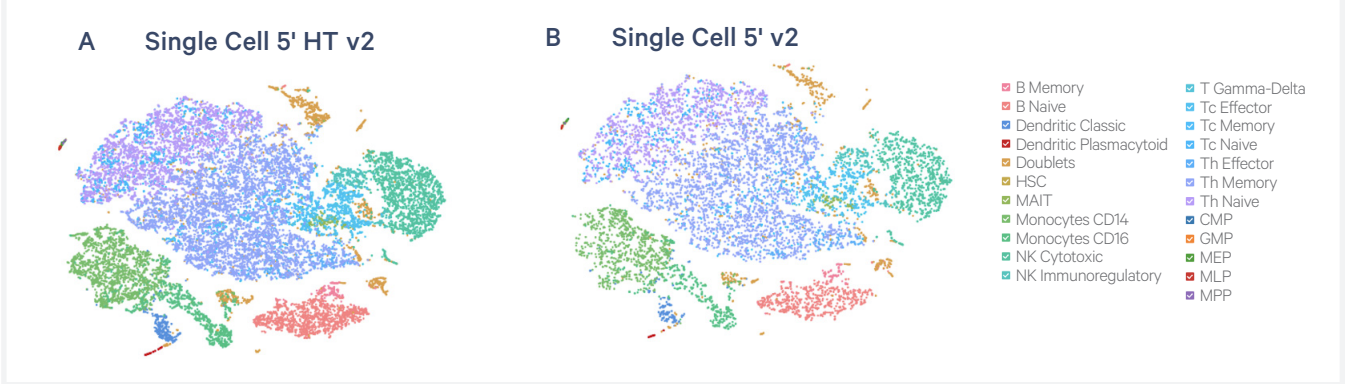


Figure 7. Cell type annotation using both 5' gene expression and cell surface protein labeling data for Cell 5' HT v2 (A) and standard Single Cell 5' v2 assays (B) is shown.

Data Highlight Contd.

Comparable Cell Types Abundance

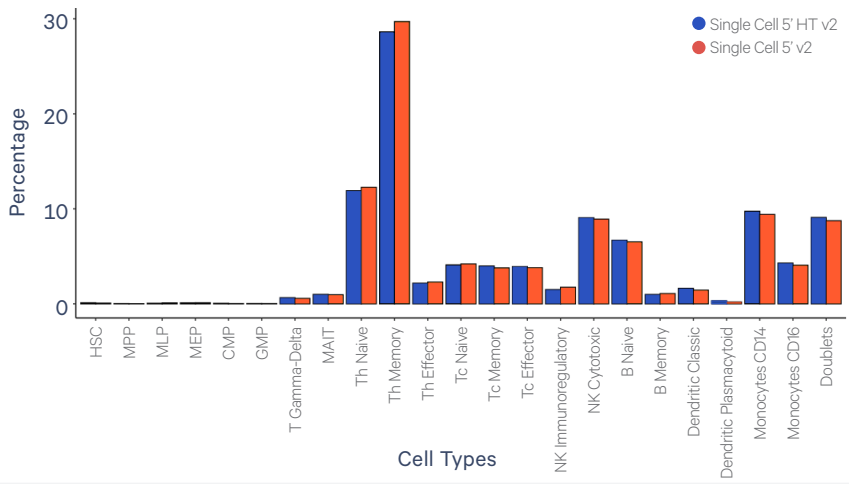


Figure 8. Comparable abundance of cell types was observed between the Single Cell 5' HT v2 and the standard Single Cell 5' v2 data.

Comparable V(D)J Clonotypes

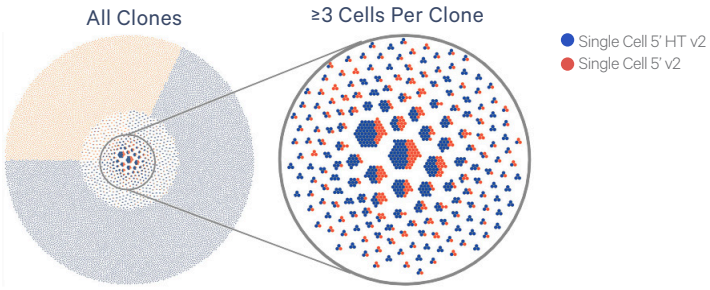


Figure 9. Shown are honeycomb plots in which each T-cell receptor clonotype is represented by a cluster of hexagons colored according to the gene expression assay (standard or HT) used. Each hexagon represents an individual T cell with a TCR specific sequence. T-cell repertoires are comparable between the data derived from Single Cell 5' HT v2 (blue hexagons) and standard Single Cell 5' v2 (red hexagons) assays. The scale of HT enables detection of rare TCR clonotypes.

Expanded V(D)J Clonotypes & Rare T-Cell Receptors

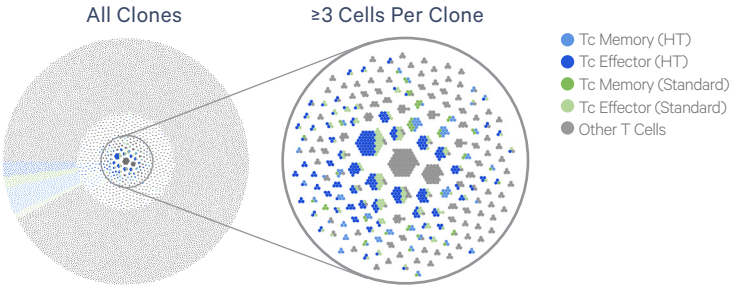


Figure 10. Shown are honeycomb plots in which memory and effector T-cell receptor clonotypes are represented by a cluster of hexagons colored according to the gene expression assay (standard or HT) used. Each hexagon represents an individual T cell with a TCR specific sequence. Enhanced TCR clonality was observed in data derived from Single Cell 5' HT v2 (blue hexagons) compared to the standard Single Cell 5' v2 assays (green hexagons).

Conclusions

The data generated using the Single Cell 5' HT v2 and the standard Single Cell 5' v2 assays are comparable in terms of library complexity, mapping rates, gene expression, and cell surface protein based cell detection. Additionally, the scale of the Single Cell 5' HT v2 assay enables detection of rare V(D)J clonotypes.

Chromium Next GEM Single Cell 5' HT v2 – Product List & Documents

Product list for generating Chromium Single Cell 5' Gene Expression Libraries using the high throughput Single Cell 5' HT v2 assay :		
Reagent Kits	**Reactions**	**Part Number (PN)**
Chromium Next GEM Single Cell 5' HT Kit v2	8 rxns	1000374
	48 rxns	1000356
Chromium Next GEM Chip N Single Cell Kit	16 rxns	1000375
	80 rxns	1000357
Chromium Single Cell Human TCR Amplification Kit	16 rxns	1000252
Chromium Single Cell Human BCR Amplification Kit	16 rxns	1000253
Chromium Single Cell Mouse TCR Amplification Kit	16 rxns	1000254
Chromium Single Cell Mouse BCR Amplification Kit	16 rxns	1000255
Dual Index Kit TT Set A	96 rxns	1000215
Additional Kits for Feature Barcode technology Protocols	**Reactions**	**Part Number (PN)**
5' Feature Barcode Kit	16 rxns	1000256
Dual Index Kit TN Set A	96 rxns	1000250
Instrument		
Chromium X Upgrade Kit	-	1000331 *(12 month warranty)* 1000332 *(24 month warranty)*
User Guides		
Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (Dual Index)	CG000423	
Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein	CG000424	
Chromium X series (X/iX) with Readiness Test	CG000396	
Software		
Cell Ranger Analysis Pipeline (DOWNLOAD)		
Loupe Cell Browser (DOWNLOAD)		

Document Revision Summary

Document Number	CG000425
Title	Chromium Next GEM Single Cell 5' HT v2: Reagents, Workflow & Data Overview
Revision	Rev A
Revision Date	August 2021

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