TECHNICAL NOTE

Chromium Next GEM Single Cell 5' v2 (Dual Index): Reagent, Workflow & Performance Updates

Introduction

The Chromium Single Cell Immune Profiling Solution provides a comprehensive, scalable solution to simultaneously examine the cellular context of the immune system and the immune repertoire of hundreds to tens of thousands of cells on a cell-by-cell basis. The product has been upgraded to version 2 (v2). The updated biochemistry includes a substantial increase in sensitivity, with up to a 60% increase in genes and transcripts detected compared to v1 and v1.1. The new Chromium Single Cell Immune Profiling Solution maintains a low doublet rate (0.8% per 1,000 cells) and industry-leading high cell recovery efficiency of up to 65%. This document highlights the key changes in Chromium Next GEM Single Cell 5' v2 reagents, workflow updates for generating and analyzing Illumina-ready sequencing libraries, and also provides a comparison of assay performance between v1.1 and v2. Refer to the Chromium Next GEM Single Cell 5' v2 (Dual Index) User Guides for the complete protocol.

Chromium Next GEM Single Cell 5' v2 Workflow

VERSION SPECIFIC

The Chromium Next GEM Single Cell 5' v2 workflow (Figure 1) is similar to the Chromium Next GEM Single Cell V(D)J v1.1 workflow, with a few specific updates that are indicated by a "Version Specific" icon adjacent to the updated steps in the Chromium Next GEM Single Cell 5' v2 (Dual Index) User Guides.

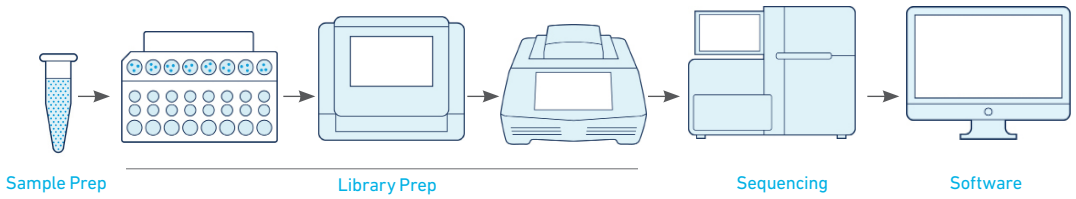


Figure 1. Chromium Next GEM Single Cell 5' v2 workflow.

Chromium Next GEM Chip K

The Chromium Next GEM Chip K is assembled in the Chromium Next GEM Secondary Holder. GEMs are generated by combining a Master Mix containing cells and enzymes, barcoded Single Cell VDJ 5' Gel Beads, and Partitioning Oil onto the Chromium Next GEM Chip K (Figure 2).

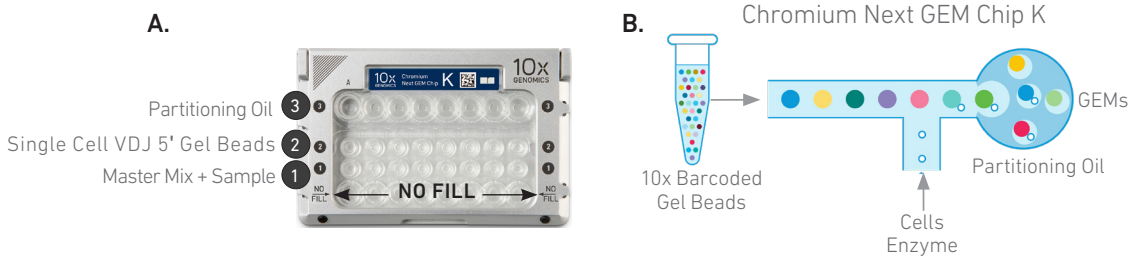


Figure 2. Chromium Next GEM Chip K assembled in a Chromium Next GEM Secondary Holder (A). GEM generation in Chromium Next GEM Chip K (B).

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

Reagents & Workflow Updates

Sample Prep

Recommendations for preparing single cell suspensions are unchanged for Chromium Next GEM Single Cell 5' v2 (Dual Index) protocols.

Library Prep

Protocol Steps	Single Cell V(D)J v1	Next GEM Single Cell V(D)J v1.1	Next GEM Single Cell 5' v2
Reagent Kits			
10x Genomics Reagents	Chromium Single Cell V(D)J Reagent Kits	Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1	Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index)
	Chromium Single Cell 5' Feature Barcode Library Kit	Chromium Single Cell 5' Feature Barcode Library Kit	5' Feature Barcode Kit
	Chromium i7 Multiplex Kit Chromium i7 Multiplex Kit N, Set A	Single Index Kit T Set A Single Index Kit N Set A	Dual Index Kit TT Set A Dual Index Kit TN Set A
	Chromium Chip A Single Cell Kit	Chromium Next GEM Chip G Single Cell Kit	Chromium Next GEM Chip K Single Cell Kit
GEM Generation & Barcoding			
GEM-RT Reagents in Master Mix	RT Reagent Mix Poly-dT RT Primer Additive A RT Enzyme Mix B	RT Reagent B Poly-dT RT Primer Additive A RT Enzyme Mix B	RT Reagent B Poly-dT RT Primer Reducing Agent B RT Enzyme C
Master Mix volume	68.3 µl	37.2 µl	36.3 µl (updated Master Mix volume)
Cell Suspension Volume Table	-	-	Updated volumes
Gel Beads	Single Cell 5' Gel Beads	Single Cell VDJ 5' Gel Beads v1.1	Single Cell VDJ 5' Gel Bead
Chip Loading			
Master Mix + Sample **Gel Beads** **Partitioning Oil**	90 µl - row labeled 1 40 µl - row labeled 2 270 µl - row labeled 3	70 µl - row labeled 1 50 µl - row labeled 2 45 µl - row labeled 3	70 µl - row labeled 1 50 µl - row labeled 2 45 µl - row labeled 3
Chromium Controller			
Firmware Version	2.0 or higher	4.0 or higher	4.0 or higher
Run time	~6.5 min	~18 min	~18 min
GEM Recovery			
	From top row labeled "NO FILL"	From top row labeled "3"	From top row labeled "3"

Library Prep contd.

Protocol Steps	Single Cell V(D)J v1	Next GEM Single Cell V(D)J v1.1	Next GEM Single Cell 5' v2
cDNA Amplification & QC			
cDNA Amplification Mix for Feature Barcode technology	SC5' Feature cDNA Primers Amplification Master Mix	SC5' Feature cDNA Primers Amplification Master Mix	Feature cDNA Primers 4 Amp Mix
cDNA Amplification Mix	cDNA Additive cDNA Primer Mix Amplification Master Mix	cDNA Additive cDNA Primer Mix Amplification Master Mix	cDNA Primers Amp Mix
V(D)J Amplification from cDNA			
V(D)J Amplification 1 & 2 Reaction Mix	Amplification Master Mix cDNA Additive Enrichment Primers	Amplification Master Mix cDNA Additive Enrichment Primers	Amp Mix TCR/BCR Amplification Primers
Primer Volume in V(D)J Amplification Reaction Mix 1 & 2	5 & 5 µl	5 & 5 µl	48 & 15 µl
Library Construction			
Fragmentation Mix	Fragmentation Buffer Fragmentation Enzyme Blend	Fragmentation Buffer Fragmentation Enzyme Blend	Fragmentation Buffer Fragmentation Enzyme
Adaptor Ligation Mix	Ligation Buffer DNA Ligase Adaptor Mix	Ligation Buffer DNA Ligase Adaptor Mix	Ligation Buffer DNA Ligase Adaptor Oligos
Sample Index PCR	Amplification Master Mix SI-PCR Primer	Amplification Master Mix SI-PCR Prime	Amp Mix
Sample Index Plate	Chromium Index Plate T Chromium Index Plate N Set A	Single Index Plate T Set A Single Index Plate N Set A	Dual Index Plate TT Set A Dual Index Plate TN Set A

Sequencing

Sequencing Type			
	Paired-end, single indexing	Paired-end, single indexing	Paired-end, dual indexing
Sequencing Read			
	Read 1: 26 cycles i7 Index: 8 cycles Read 2: 91cycles	Read 1: 26 cycles i7 Index: 8 cycles Read 2: 91cycles	Read 1: 26 cycles i7 Index: 10 cycles i5 Index: 10 cycles Read 2: 90 cycles

Performance Updates

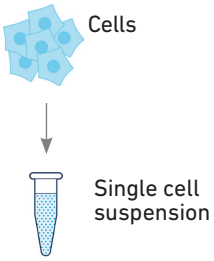
To assess the performance of the Chromium Single Cell Immune Profiling v2 solution, cells originating from the same single cell suspensions were processed with both the Chromium Single Cell 5' v1.1 and the Chromium Single Cell 5' v2 workflows for Gene Expression, TCR, BCR, and Cell Surface Protein library generation. This comparison was performed across several complex sample types. Significant performance gains were seen across all sample types tested. An overview of the methods and the results is described in the following sections.

Methods Overview

Single cell suspensions were prepared from following four sample types:

- Unstimulated human peripheral blood mononuclear cells (PBMCs)
- Mouse splenocytes
- Human melanoma dissociated tumor cells
- Human gastroesophageal dissociated tumor cells

For PBMCs and mouse splenocytes, ~1,000 were targeted. For dissociated tumor cells, 10,000 cells were targeted. PBMCs were further treated with TotalSeq-C antibodies as described in Demonstrated Protocol Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149).



	Chromium Single Cell 5' v1.1 workflow	Chromium Single Cell 5' v2 (Dual Index) workflow
Protocol	Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 with Feature Barcode technology (CG000208)	Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology (CG000330)
Libraries	V(D)J, Gene Expression, and Cell Surface Protein	V(D)J, Gene Expression, and Cell Surface Protein
Sequencing	Depth: ~30,000 raw reads per cell Cycles: Read 1–26; i7–8; Read 2–91	Depth: ~30,000 raw reads per cell Cycles: Read 1–26; i5–10; i7–10; Read 2–90
Analysis Visualization	Cell Ranger 4.0 Loupe Browser 4.1.0	Cell Ranger 4.0 Loupe Browser 4.1.0

Results Overview

Assay Sensitivity:

At the recommended sequencing read depth of 20,000 raw reads per cell, libraries prepared using v2 biochemistry show an increase in median genes per cell of up to 55%. In a human PBMC sample, ~1,750 median genes per cell at ~40,000 read pairs per cell were observed compared to ~1,110 median genes per cell observed in the v1.1 biochemistry (55% increase). Similar gains in sensitivity are also seen in more complex and challenging cell types, such as human melanoma and gastroesophageal dissociated tumor cells, and mouse splenocytes (Figure 3). More median genes per cell at lower sequencing depth (~20,000 raw read pairs per cell) are detected in samples prepared with the Chromium Single Cell Immune Profiling v2 compared to the same samples sequenced at much higher sequencing depth (40,000 raw read pairs per cell) and prepared with the v1.1 biochemistry.

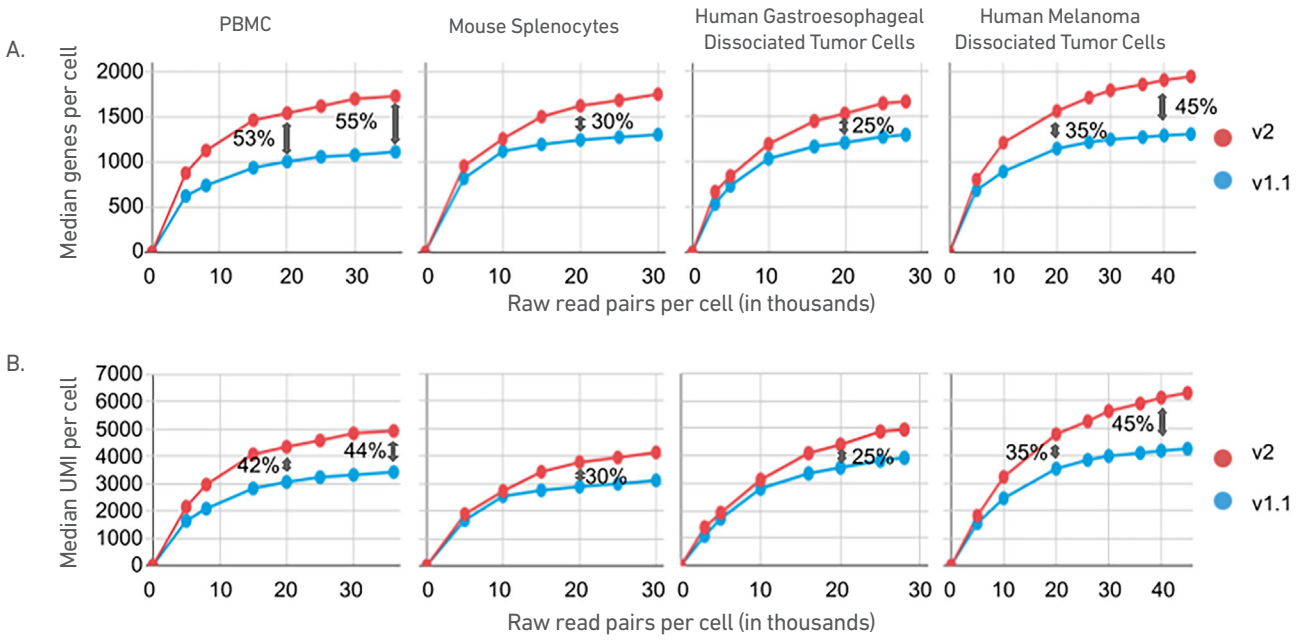
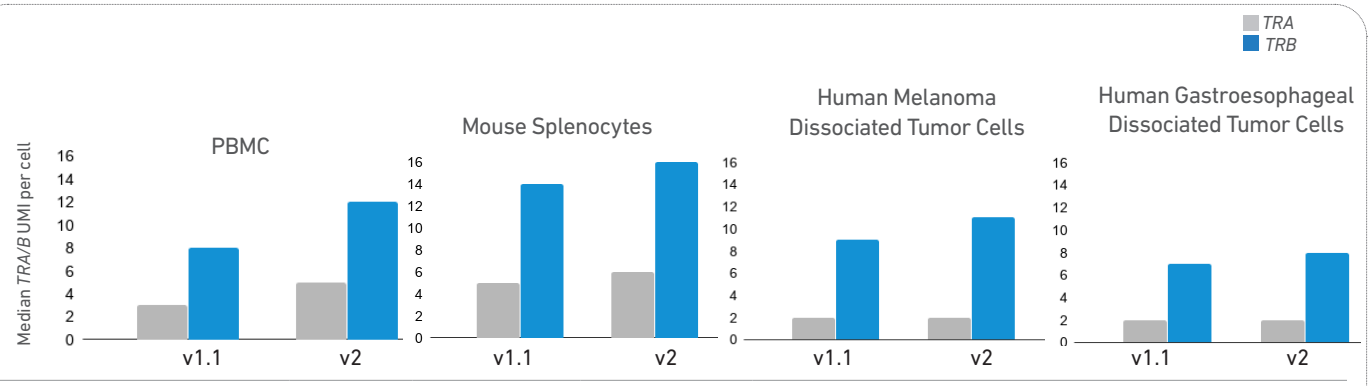


Figure 3. Gene expression saturation curves derived from Chromium Single Cell v1.1 and v2 sequencing data. A. Median genes per cell **B.** Median UMI counts per cell. Percentage increase in median genes per cell or median UMIs per cell is provided as 20,000 raw read pairs per cell and 40,000 raw read pairs per cell.

V(D)J Sequences Recovered:

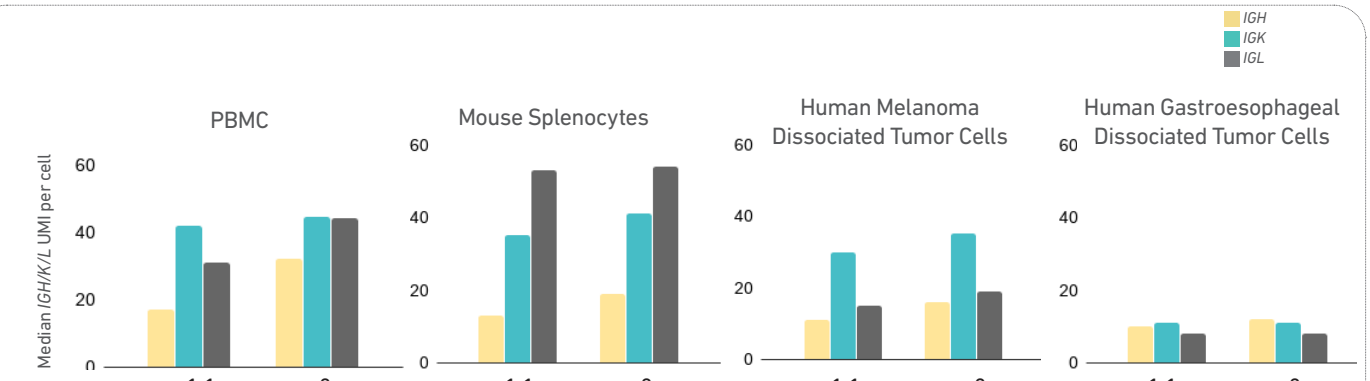
A key feature of the Chromium Single Cell Immune Profiling Solution is the ability to combine gene expression data, with full-length, paired V(D)J receptor sequences for T and B cells at single cell resolution, allowing for a more complete understanding of the adaptive immune system. The assay sensitivity improvements also allow for improved recovery of V(D)J receptor transcripts here, with an up to 77% increase in median UMIs for *IGH/K/L* genes per cell, and an up to 50% increase in median *TRA/TRB* UMIs per cell, when evaluating the v2 biochemistry against v1.1 (Figure 4). Together, an improved V(D)J detection rate as measured by both an increase in the total number of V(D)J UMIs, as well as an increased number of cells with a productive V-J spanning pair is observed.

A.



	PBMC		Mouse Splenocytes		Human Melanoma Dissociated Tumor Cells		Human Gastroesophageal Dissociated Tumor Cells	
Pairing rate	84.7%	86.3%	81.8%	87.5%	68.5%	70.2%	61.5%	62.0%
Cells with productive spanning pair	344	416	121	175	1,069	1,444	919	1,141

B.



	PBMC		Mouse Splenocytes		Human Melanoma Dissociated Tumor Cells		Human Gastroesophageal Dissociated Tumor Cells	
Pairing rate	96.7%	97.6%	94.1%	94.4%	92.8%	93.8%	90.7%	90.8%
Cells with productive spanning pair	59	81	589	747	3,903	5,293	4,268	5,038

Figure 4. Median V(D)J sequences recovered per cell for *TRA/TRB* (A) and *IGH/IGK/IGL* (B) transcripts across samples processed with v1.1 or v2 biochemistry. In all samples, an improvement in V(D)J detection rate is observed as indicated by increased number of cells with productive V-J spanning pair.

Cell Populations Recovered:

The relative proportion of cellular populations identified when comparing replicate PBMC samples are the same; this was consistent across different biochemistries (v1.1 and v2), users, targeted cells recovered, and technical replicates (Figure 5). The same holds true when analyzing cell types based on cell surface protein expression, with similar cell populations recovered in both biochemistries (Figure 6).

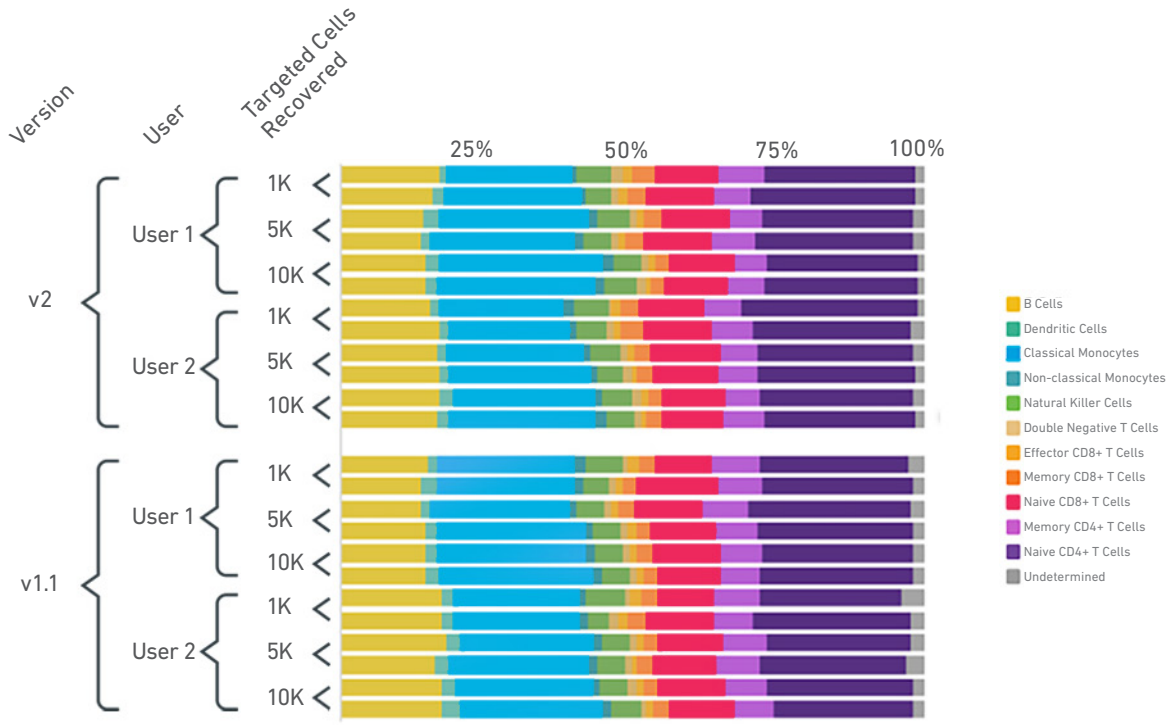


Figure 5. Unstimulated PBMC samples were tested across different biochemistries, different users, and different targeted cells recovered. Each experiment also included a technical replicate. Expected cell populations in similar frequencies were recovered across all experimental conditions.

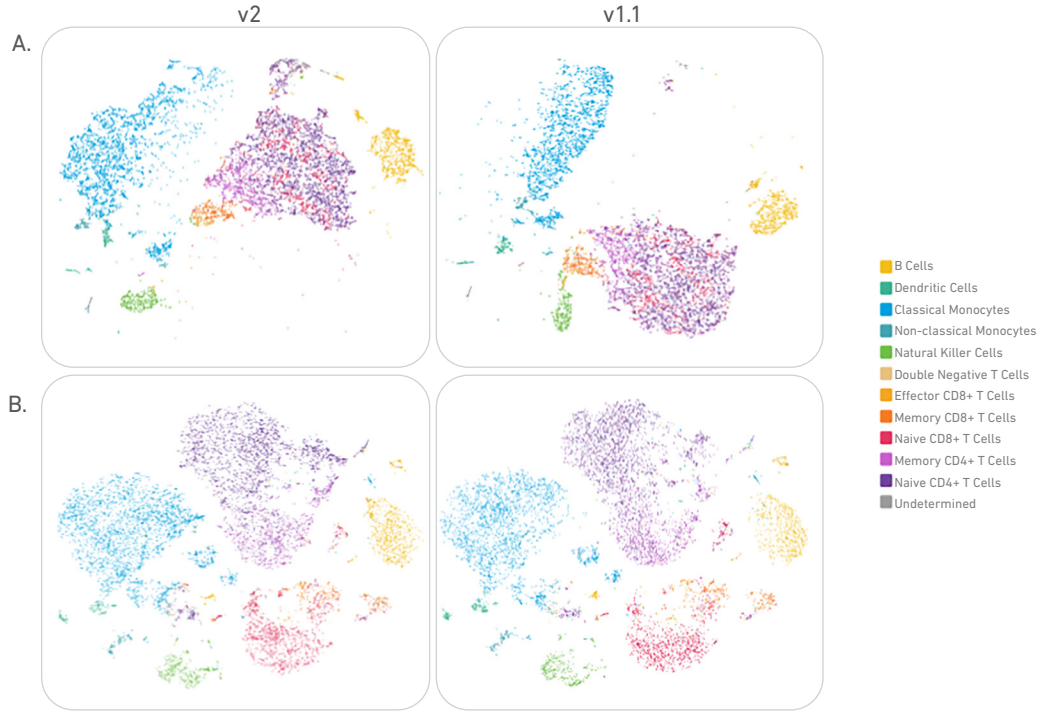


Figure 6. Unsupervised clustering and automated annotation of the same PBMC sample split and processed on v2 and v1.1 biochemistry. 10,000 cells were targeted for both workflows. Top row **(A)** shows clustering based on gene expression while bottom row **(B)** shows clustering based on cell surface protein labeling. Same cell types with similar frequencies were recovered across both biochemistries.

V(D)J Clonotypes:

Increased numbers of *TRA/TRB* and *IGH/K/L* UMIs are identified with the v2 biochemistry, which in some samples, results in an increased number of cells with a productive V-J spanning pair. This, in combination with the increased number of cells detected leads to a substantial improvement in the amount of useful data that is generated with the v2 workflow. Clonal frequencies were in expected range for a PBMC population from a healthy donor (Figure 7).

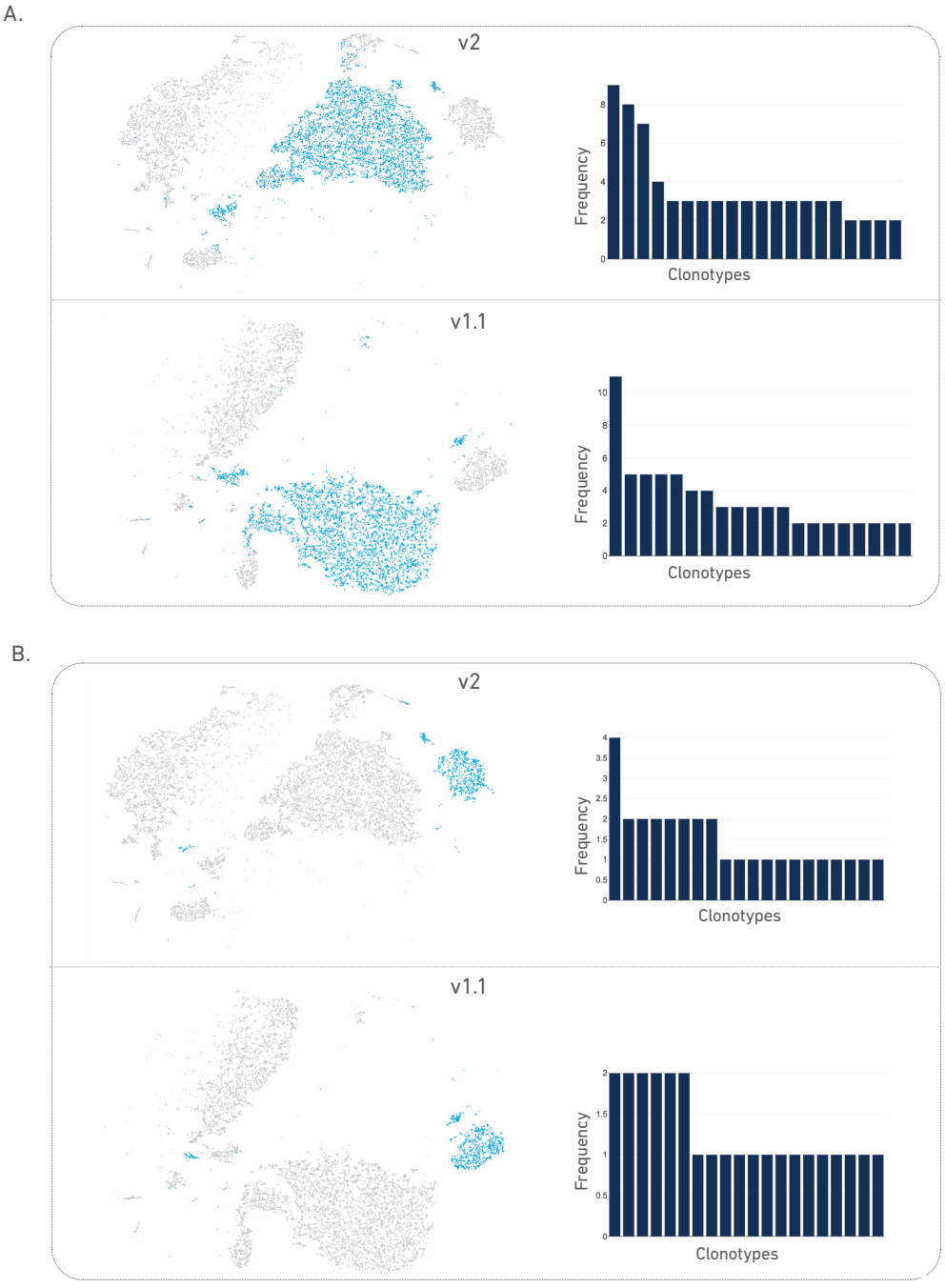


Figure 7. V(D)J clonotypes were imported into Loupe Browser for visualization. A. TCR transcripts detected in v2 and v1.1. **B.** BCR transcripts detected in v2 and v1.1.

Cells highlighted in blue are cells that were called as either T or B cells by Cell Range V(D)J based on their expression of TCR or BCR transcripts. The same cells called as T or B cells based on gene expression are also called as T or B cells by their expression of V(D)J transcripts. As the sample is from a healthy donor, clonotypes in both T and B cell populations are extremely diverse. Same T cell clonotype is recovered in both v1.1 and v2 (TRAV26-2:TRAJ24:TRAC, TRBV7-3:TRBJ1-1:TRBC1).

Conclusion

In conclusion, the Chromium Single Cell Immune Profiling v2 shows substantial gains in assay sensitivity across multiple sample types. These considerable improvements in performance allow for an expanded understanding of sample complexities at both the gene expression and receptor sequence levels and allow for multiomic interrogation of complex samples with single cell resolution.

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

Product list for generating Chromium Single Cell Libraries:

REAGENT KITS	REACTIONS	PART NUMBER (PN)
Chromium Next GEM Single Cell 5' Kit v2	16 rxns 4 rxns	1000263 1000265
Library Construction Kit	16 rxns	1000190
Chromium Single Cell Human TCR Amplification Kit Chromium Single Cell Human BCR Amplification Kit Chromium Single Cell Mouse TCR Amplification Kit Chromium Single Cell Mouse BCR Amplification Ki	16 rxns 16 rxns 16 rxns 16 rxns	1000252 1000253 1000254 1000255
Chromium Next GEM Chip K Single Cell Kit	48 rxns 16 rxns	1000286 1000287
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 Browser (DOWNLOAD)		
Loupe V(D)J Browser (DOWNLOAD)		
DOCUMENTS		
User Guide : Chromium Next GEM Single Cell 5' v2 (Dual Index) (CG000331)		

If using Single Cell 5' Reagent Kits v2 (Dual Index) protocols with Feature Barcode technology, the following products are required in addition to all the products listed above. Refer to the indicated documents for specific guidance.

5' Feature Barcode Library Kit	16 rxn	1000256
Dual Index Kit TN Set A	96 rxns	1000250
DOCUMENTS		
User Guide : Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping (CG000330) Demonstrated Protocol : Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149) Demonstrated Protocol : Cell Labeling with Dextramer Reagents for Single Cell RNA Sequencing Protocols (CG000203)		

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