

# Chromium Next GEM Single Cell ATAC v2: Reagents, Workflow & Data Overview

## Introduction

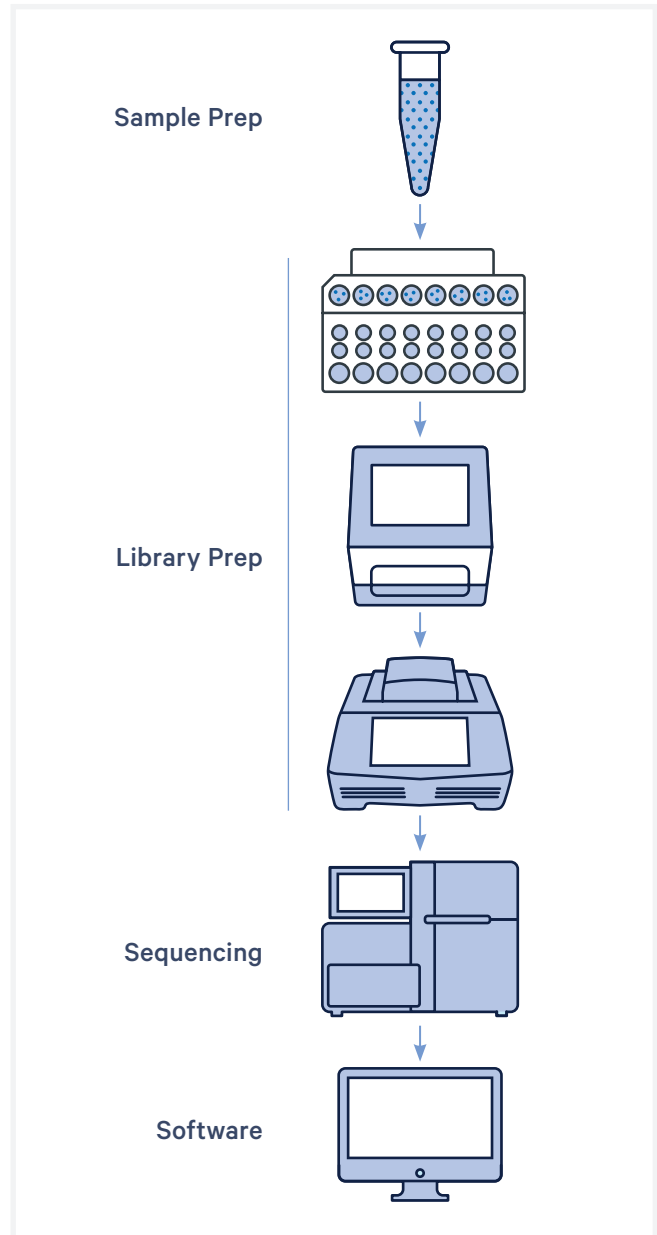
The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. This Technical Note highlights sample preparation, reagents, and workflow specifics for Single Cell ATAC v2, along with information about data analysis. A comparison of representative data derived from Single Cell ATAC v2 versus both Single Cell ATAC v1.1 and Chromium Single Cell Multiome ATAC + Gene Expression assays is also presented.

Refer to the Chromium Next GEM Single Cell ATAC Reagent Kits v2 User Guide for the complete protocol.

## Chromium Next GEM Single Cell ATAC v2 Workflow

Chromium Next GEM Single Cell ATAC v2 workflow is similar to the Chromium Next GEM Single Cell ATAC v1.1 workflow, with specific updates that are indicated by a "version specific" icon adjacent to the updated steps in the Single Cell ATAC v2 User Guide (see [Product List & Documents](#) section for link to User Guides).

Figure 1 provides a high level overview of the Single Cell ATAC v2 workflow.



**Figure 1.** Chromium Next GEM Single Cell ATAC v2 workflow.

The key differences between the Single Cell ATAC v1.1 and the Single Cell ATAC v2 assays are presented in the table below. Refer to the relevant User Guides for complete information.

Single Cell ATAC v1.1		Single Cell ATAC v2
<b>10x Genomics Reagents</b>		
	Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 16 rxn & 4 rxn kits	Chromium Next GEM Single Cell ATAC Reagent Kits v2 16 rxn & 4 rxn kits <i>(See <a href="#">Product List &amp; Documents</a> for details)</i>
<b>Transposition</b>		
Nuclei Buffer	1 tube 20X Nuclei Buffer	2 tubes 20X Nuclei Buffer
Transposition Enzyme	ATAC Enzyme	ATAC Enzyme B
Transposition Time	60 minute transposition	30 minute transposition
<b>GEM Generation &amp; Barcoding</b>		
Gel Beads	Single Cell ATAC Gel Bead v1.1	Single Cell ATAC Gel Bead v2
<b>Chip Loading</b> <i>(loading configurations &amp; volumes unchanged)</i>		
	Chip H	Chip H
<b>Instrument</b>		
Firmware Version	4.0 (for Chromium Controller only) or higher	4.0 (for Chromium Controller only) or higher
Instrument Compatibility	Chromium Controller & Chromium X/iX	Chromium Controller & Chromium X/iX
Run Time	Run time ~18 min	Run time ~18 min
<b>Library Construction</b>		
SI-PCR Cycles	11, 10, or 9 cycles, depending on cell load	9, 8, or 7 cycles, depending on cell load
<b>Sequencing</b>		
	Recommendations on sequencing libraries generated using the Chromium Next GEM Single Cell ATAC Reagents Kits v2 protocol are the same as sequencing recommendations for libraries generated using the Chromium Single Cell ATAC Reagent Kits v1.1 protocol.	
<b>Software</b>		
Cell Ranger ATAC	Cell Ranger ATAC v2.0 or later	Cell Ranger ATAC v2.1 (with chemistry batch detection / correction)

## Results

The representative Data Highlight provides a Methods Overview along with comparison of key results derived from human GM12878 cells mixed 1:1 with mouse EL4 cells, in addition to human PBMCs and mouse E18 brain cells. The libraries were generated using the specified Single Cell ATAC v1.1, Single Cell ATAC v2, and Single Cell Multiome ATAC + Gene Expression reagents and protocols, were sequenced, and the data were analyzed and visualized using Cell Ranger ATAC v2.1 and Loupe Browser. The results shown in Figures 2-6 clearly demonstrate that the Single Cell ATAC v2 assay

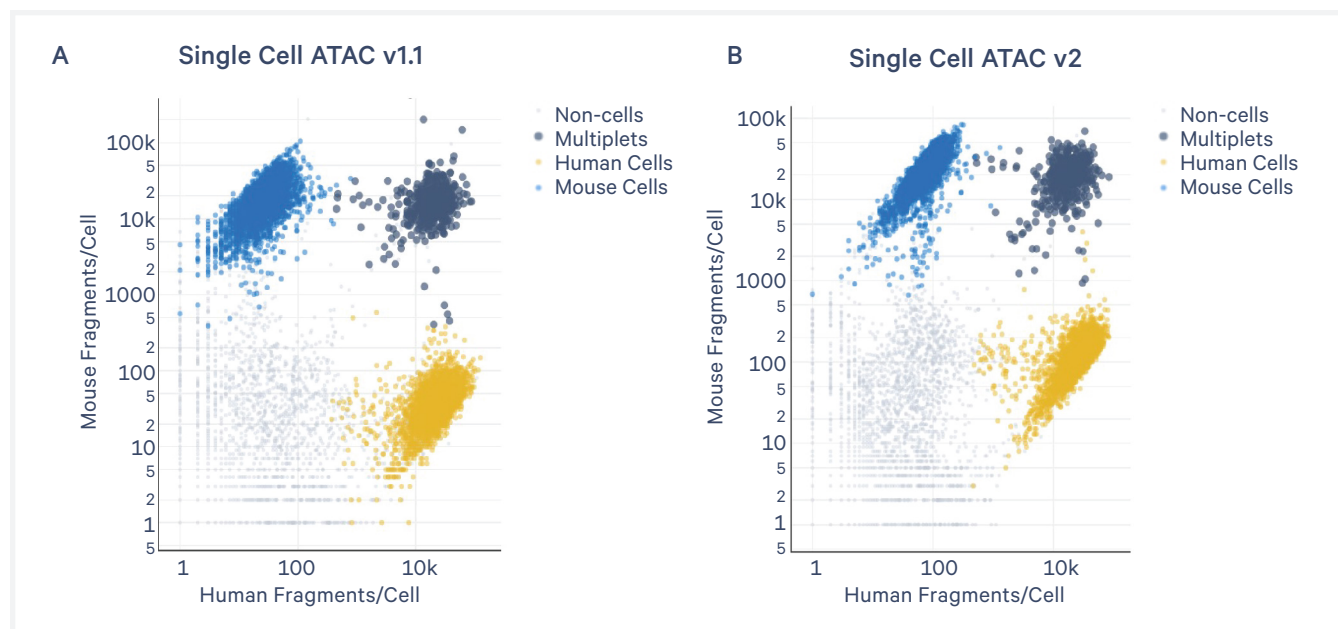
yields improved data in terms of library complexity and sensitivity, as well as peaks detected, when compared to Single Cell ATAC v1.1. Multiplet and mapping rates were comparable. Additionally, Cell Ranger ATAC v2.1 offers batch correction capabilities that remove variability in chromatin accessibility profiles between the Single Cell ATAC v1.1 and v2 chemistries. The results shown in Figures 7-9 highlight the improved library complexity and sensitivity in Single Cell ATAC v2 compared to the Single Cell Multiome ATAC + Gene Expression assay.

## Data Highlight - Single Cell ATAC v1.1 vs. ATAC v2 Data Comparison

### Methods Overview

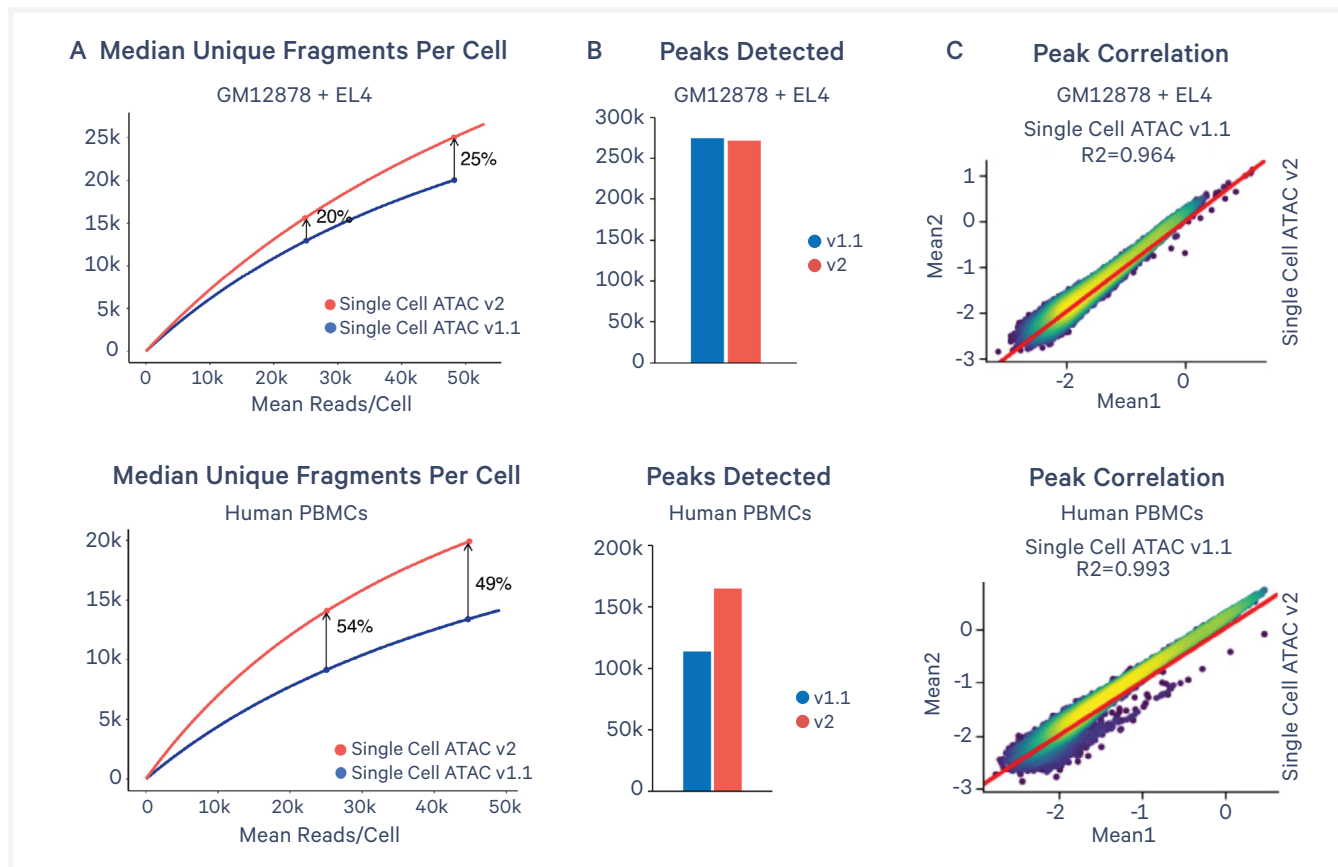
Human GM12878 (Coriell Institute), mouse EL4 (ATCC), and human PBMC (AllCells) cells were thawed and lysed according to the Nuclei Isolation for Single Cell ATAC Sequencing Demonstrated Protocol (CG000169). Nuclei from the GM12878 and EL4 cell lines were hand-mixed at a 1:1 ratio. All nuclei were then processed using Single Cell ATAC v1.1 (CG000209) and Single Cell ATAC v2 (CG000496) assays (10,000 nuclei targeted per replicate). The chips were run on Chromium X followed by library preparation, sequencing, and data analysis as described in the respective User Guides.

### Multiplet Rate



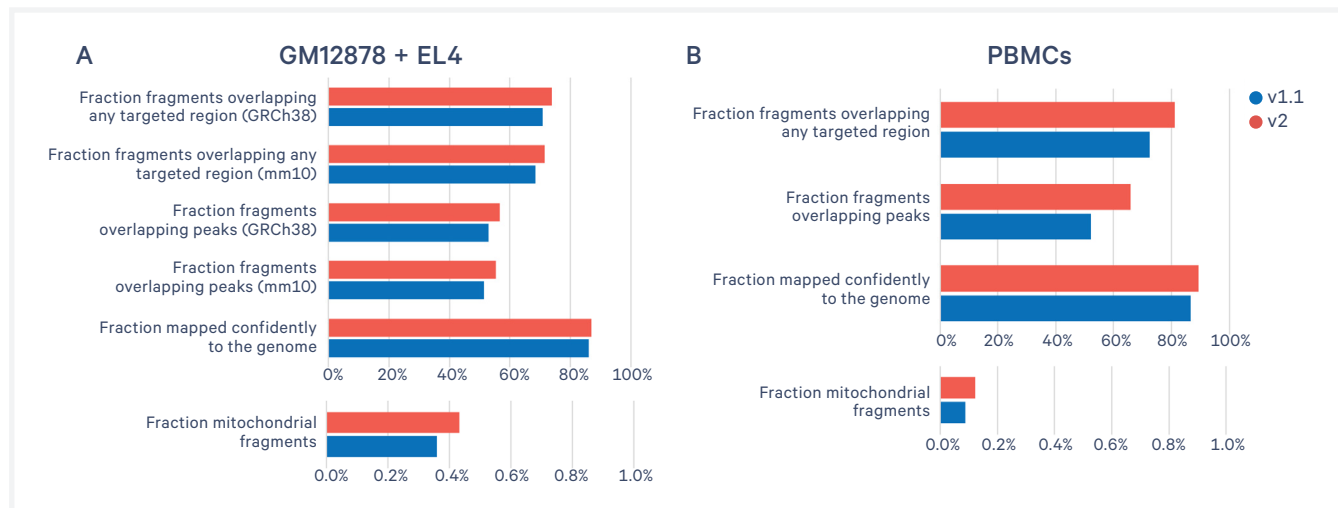
**Figure 2.** Scatter plot of the number of fragments mapped to the human and mouse genome detected in a mixture of human GM12878 and mouse EL4 cells. Cell barcodes mapping to human (yellow), mouse (blue) or both (multiplets, dark grey), are shown for (A) Single Cell ATAC v1.1 (981 multiplets in 10,672 cells detected, ~0.8% multiplets per 1,000 cells) compared to Single Cell ATAC v2 (B) assay (939 multiplets in 10,138 cells detected, ~0.9% multiplets per 1,000 cells).

### Improved Library Complexity & Correlation



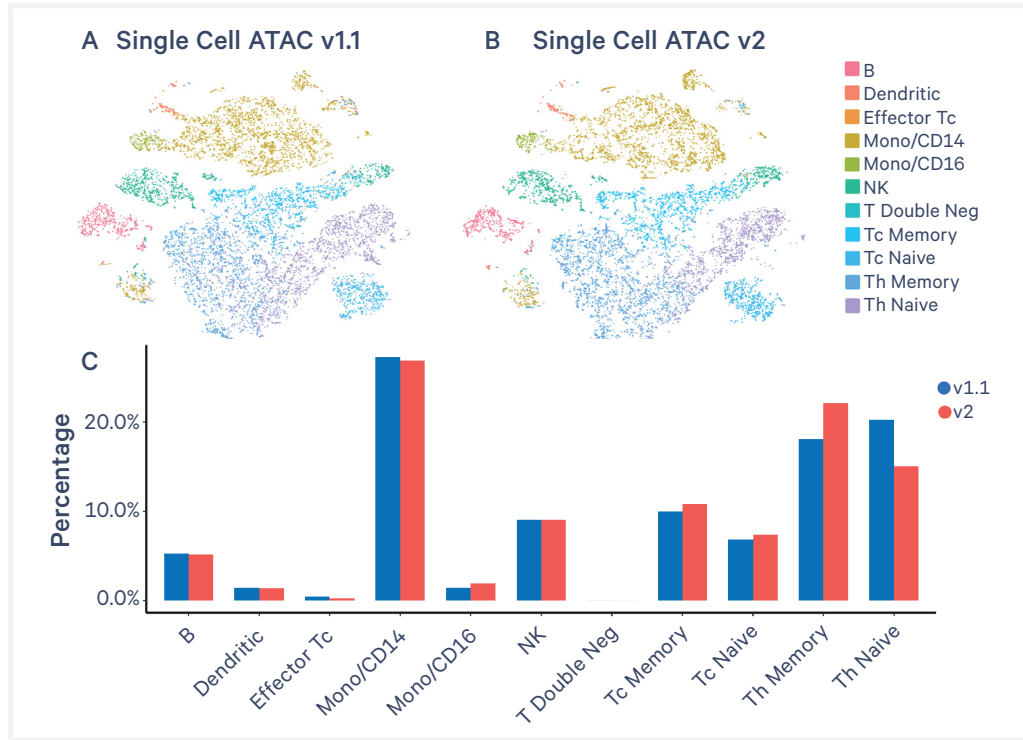
**Figure 3.** Increased median unique fragments per cell (A), increased peaks detected (B), and high peak correlation (C) was observed between the data derived from Single Cell ATAC v1.1 and Single Cell ATAC v2 assays in GM12878 + EL4 mixed cells and human PBMCs run on Chromium X.

### Comparable Read Mapping Rates



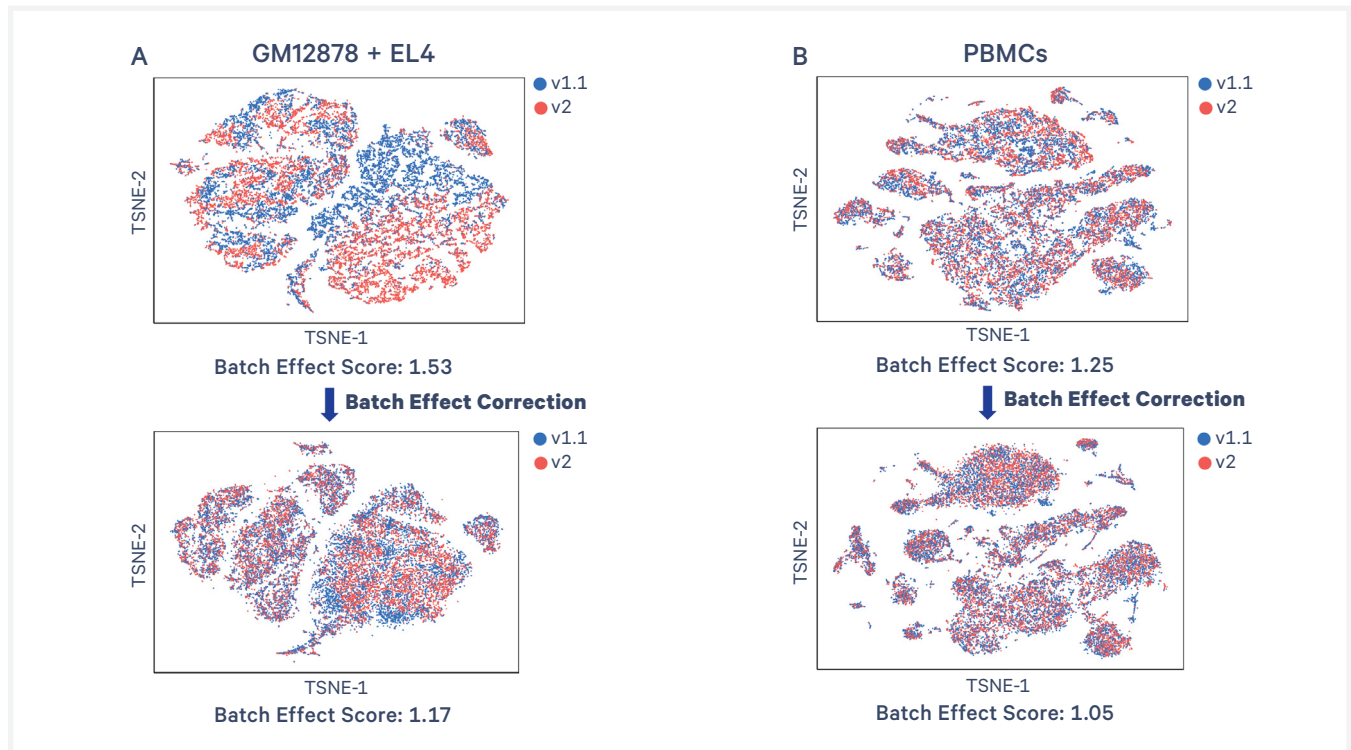
**Figure 4.** Comparable read mapping rates between the Single Cell ATAC v2 and the Single Cell ATAC v1.1 data for GM12878 + EL4 mixed cells (A) and human PBMCs (B).

### ATAC-based Cell Clustering and Cell Type Identification



**Figure 5.** Similar cell clusters and cellular populations were detected in the human PBMC cells profiled using aggregated ATAC data from the Single Cell ATAC v1.1 (A) and the Single Cell ATAC v2 assays (B). The t-SNE plots show overlapping ATAC-based cell clustering along with highly concordant cell type identification based on manual annotation. The graph shows percentage of identified cell types for both assays (C).

### Reduced Chemistry-Induced Batch Effects



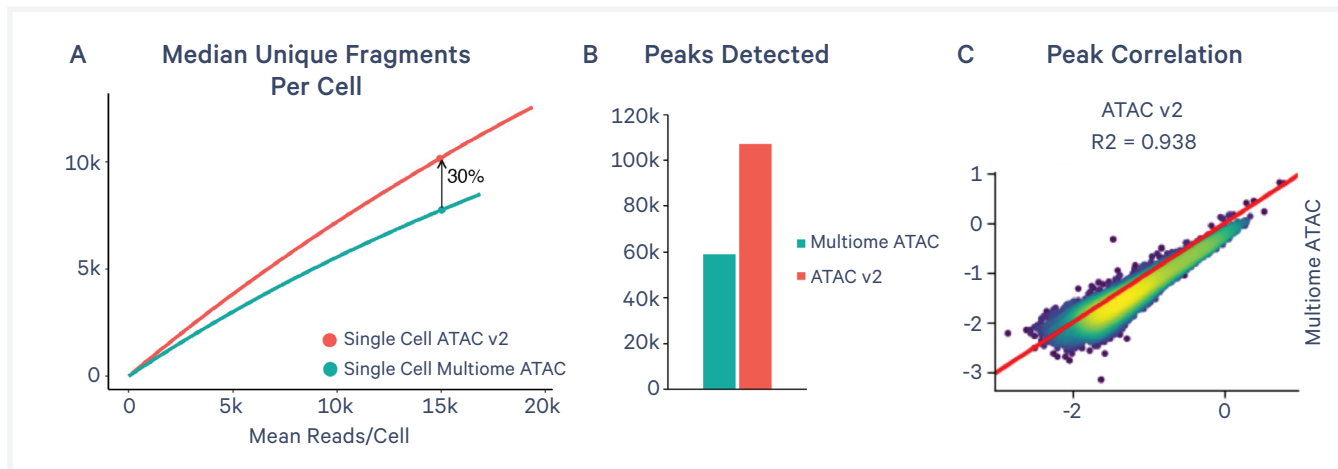
**Figure 6.** Single Cell ATAC clustering data derived from the Single Cell ATAC v1.1 (blue) and Single Cell ATAC v2 (red) assays is shown in the aggregated t-SNE plots for GM12878 + EL4 mixed cells (A) and human PBMCs (B). Cell Ranger ATAC v2.1 Chemistry Batch Correction specifically corrects for systematic variability in chromatin accessibility profiles caused by different versions of Single Cell ATAC chemistries.

## Data Highlight: Single Cell ATAC v2 vs. Single Cell Multiome ATAC Data Comparison

### Methods Overview

Nuclei were isolated from two flash frozen E18 mouse cortex (BrainBits LLC) samples. Nuclei processed for Single Cell ATAC v2 (CG000496) were isolated using the Nuclei Isolation from Mouse Brain Tissue for Single Cell ATAC Sequencing (CG000212) Demonstrated Protocol. Nuclei processed for Single Cell Multiome ATAC + Gene Expression (CG000338) were isolated using the Nuclei Isolation from Embryonic Mouse Brain Tissue for Single Cell Multiome ATAC + Gene Expression Sequencing (Document CG000366) Demonstrated Protocol. 3,000 nuclei were targeted per replicate in each assay. The chips were run on Chromium X followed by library preparation, sequencing, and data analysis as described in the respective User Guides.

### Library Complexity & Correlation



**Figure 7.** Increased median unique fragments per cell (A), increased peaks detected (B), and high peak correlation (C) was observed between the data derived from Single Cell ATAC v2 and Single Cell Multiome ATAC + Gene Expression assays in mouse E18 brain cells run on Chromium X.

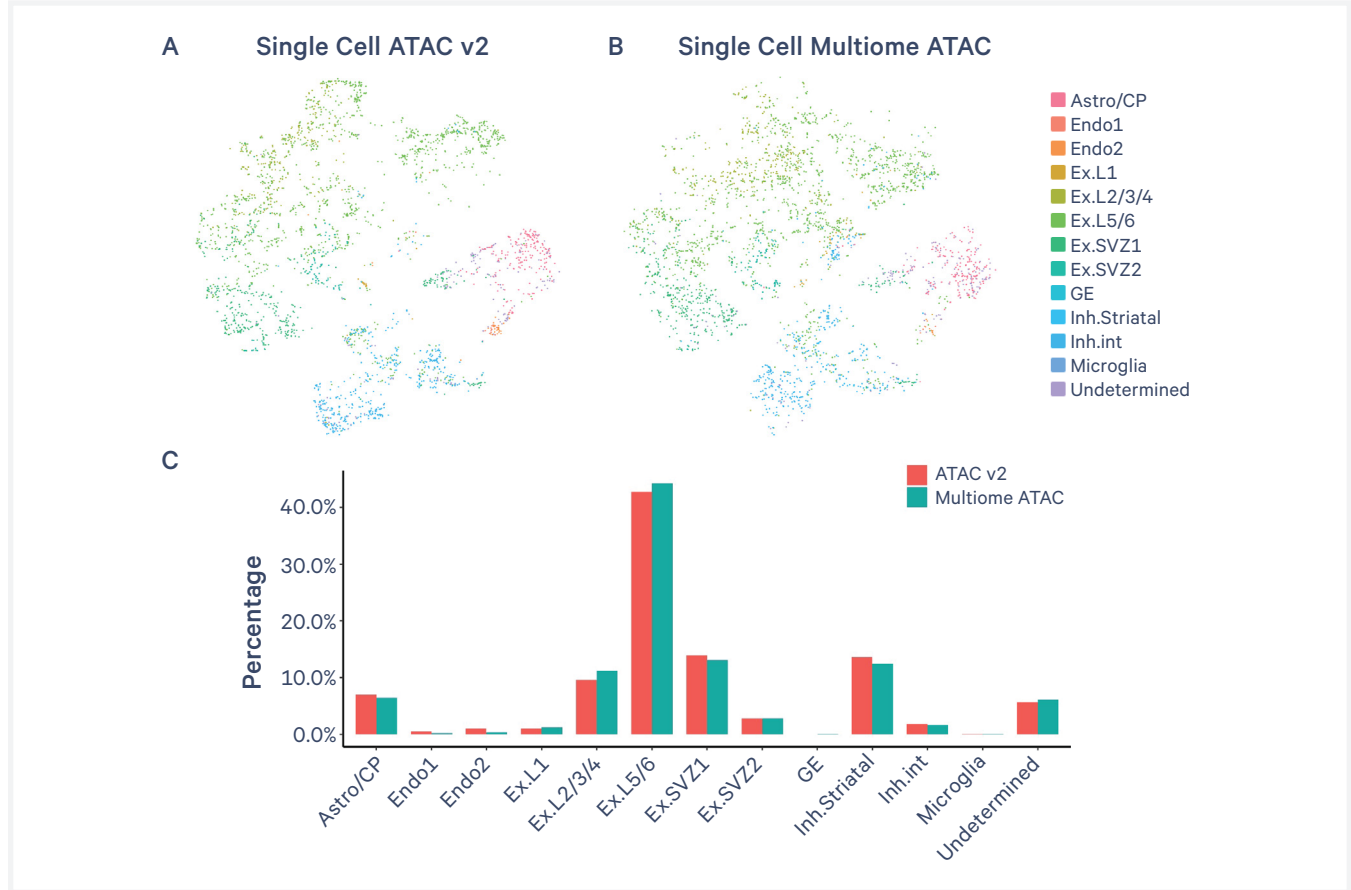
### Read Mapping Rates



**Figure 8.** Read mapping rates between the Single Cell ATAC v2 and the Single Cell Multiome ATAC data for mouse E18 brain cells.



## ATAC-based Cell Clustering and Cell Type Identification



**Figure 9.** Similar cell clusters and cellular populations were detected in the mouse E18 brain cells profiled using aggregated ATAC data from the Single Cell ATAC v2 (A) and the Single Cell Multiome ATAC + Gene Expression assays (B). The t-SNE plots show overlapping ATAC-based cell clustering along with highly concordant cell type identification based on manual annotation. The graph shows percentage of identified cell types for both assays (C).

## Conclusions

Single Cell ATAC v2 is the next generation of 10x Genomics ATAC technology for interrogating the epigenomic landscape of single cells. Compared to Single Cell ATAC v1.1, Single Cell ATAC v2 has:

- Updated biochemistry and reagent kits with improved performance
- Up to 50% increase in sensitivity
- Reduced background for improved ability to call peaks
- Comparable low multiplet rate
- Reduced total workflow time

This Technical Note specifically summarizes data demonstrating improved library complexity and sensitivity, and increased peaks detected, in the Single Cell ATAC v2 assay compared to the Single Cell ATAC v1.1 assay. The multiplet and mapping rates were comparable. The data also demonstrates improved library complexity and sensitivity in Single Cell ATAC v2 compared to the Single Cell Multiome ATAC + Gene Expression assay. Finally, the addition of batch correction technology in the Cell Ranger ATAC v2.1 software removes variabilities due to differences in the two ATAC chemistries.

## Single Cell ATAC v2 – Product List & Documents

Product list for generating Chromium Single Cell ATAC Libraries:		
Reagent Kits	Reactions	Part Number (PN)
Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v2	16 rxns	1000390
	4 rxns	1000406
Chromium Next GEM Chip H Single Cell Kit	48 rxns	1000161
	16 rxns	1000162
Single Index Kit N, Set A	96 rxns	1000212
Instrument		
Chromium Controller & Next GEM Accessory Kit		120223 <i>(12 month warranty)</i>
		120246 <i>(24 month warranty)</i>
Chromium X & Accessory Kit		1000331 <i>(12 month warranty)</i>
		1000332 <i>(24 month warranty)</i>
Chromium iX & Accessory Kit		1000328 <i>(12 month warranty)</i>
		1000366 <i>(24 month warranty)</i>
Documents		
User Guide: Chromium Next GEM Single Cell ATAC Reagent Kits v2		<a href="#">CG000496</a>
User Guide: Chromium Next GEM Single Cell ATAC Reagent Kits v1.1		<a href="#">CG000209</a>
User Guide: Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Kits		<a href="#">CG000338</a>
Demonstrated Protocol: Nuclei Isolation for Single Cell ATAC Sequencing		<a href="#">CG000169</a>
Demonstrated Protocol: Nuclei Isolation from Mouse Brain Tissue for Single Cell ATAC Sequencing		<a href="#">CG000212</a>
Demonstrated Protocol: Nuclei Isolation from Embryonic Mouse Brain Tissue for Single Cell Multiome ATAC + Gene Expression Sequencing		<a href="#">CG000366</a>
Software		
Cell Ranger ATAC v2.1 ( <a href="#">DOWNLOAD</a> )		
Loupe Browser ( <a href="#">DOWNLOAD</a> )		



## Document Revision Summary

<b>Document Number</b>	CG000533
<b>Title</b>	Chromium Next GEM Single Cell ATAC v2: Reagents, Workflow & Data Overview
<b>Revision</b>	Rev A
<b>Revision Date</b>	March 2022

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