TECHNICAL NOTE

Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Data Comparison

Introduction

The Chromium Next GEM Single Cell Multiome + Gene Expression provides a comprehensive, scalable multiomic approach for simultaneously profiling epigenomic landscape and gene expression in the same single nuclei. This technical note highlights the sensitivity and consistency at which both ATAC and gene expression data are captured by this multiomic approach as compared to the standalone Chromium Next GEM Single Cell ATAC v1.1 and Chromium Next GEM Single Cell 3' Gene Expression v3.1 assays.

Chromium Next GEM Multiome Single Cell ATAC + Gene Expression Workflow

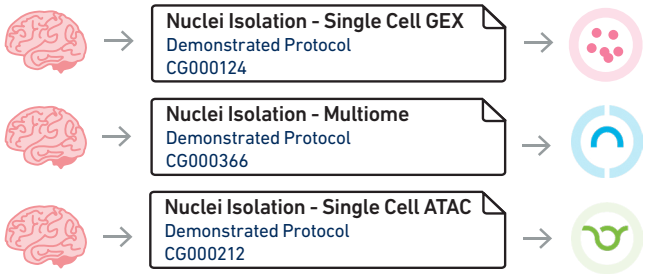
The Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (GEX) assay generates paired ATAC and GEX libraries from a single nuclei sample. The libraries can be sequenced and the data analyzed and visualized using Cell Ranger ARC and Loupe Cell Browser for assessing single cell gene expression and chromatin landscape.

Methods

Nuclei from three fresh mouse E18 brains (Brainbits, PN-C57EHCV) were isolated with the respective demonstrated protocol for each assay:

- Isolation of Nuclei for Single Cell RNA Sequencing (Document CG000124) was used for the sample run on Single Cell 3' Gene Expression v3.1.
- Nuclei Isolation from Embryonic Mouse Brain Tissue for Single Cell Multiome ATAC + Gene Expression Sequencing (Document CG000366) was used for the sample run on Single Cell Multiome ATAC + Gene Expression.
- Nuclei Isolation from Mouse Brain Tissue for Single Cell ATAC Sequencing (Document CG000212) was used for the sample run on Single Cell ATAC v1.1.

Two replicates were run on each assay targeting 5,000 nuclei recovery each.



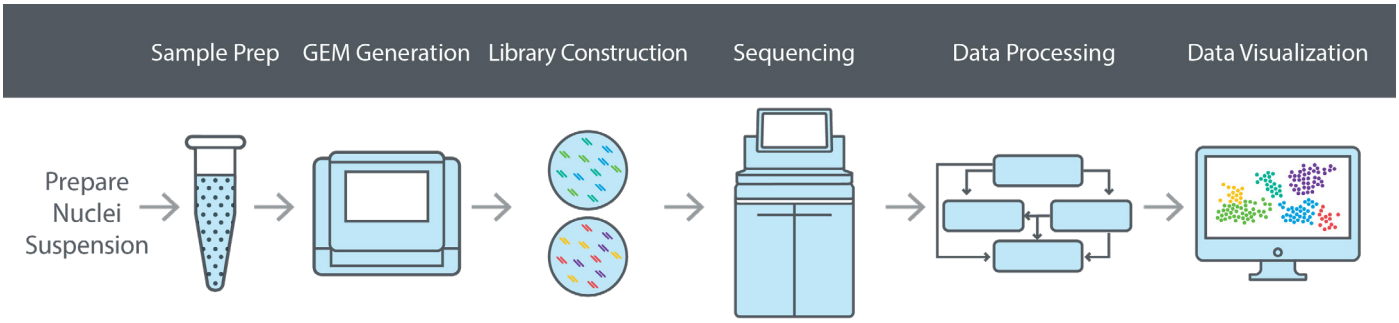


Figure 1. The Chromium Next GEM Single Cell Multiome + Gene Expression solution workflow.

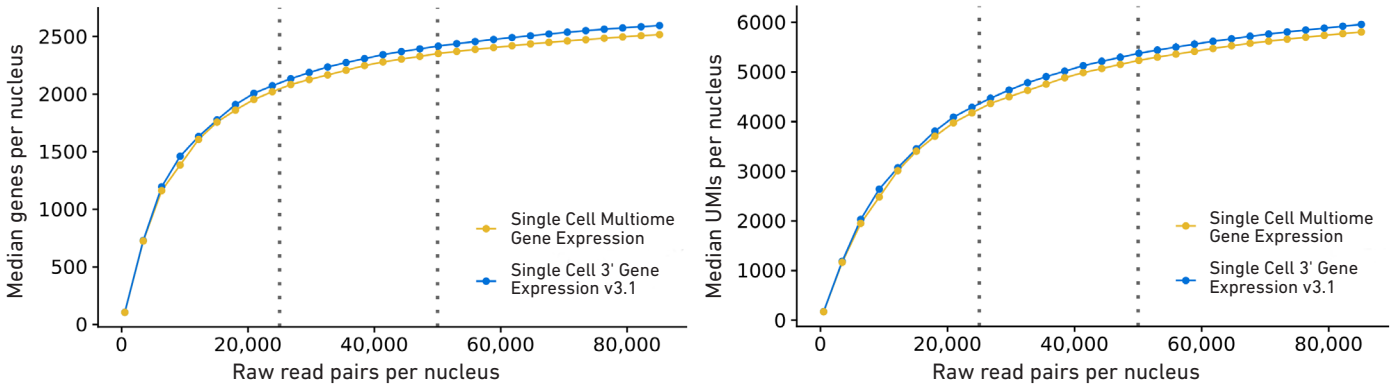
Results

Chromium Next GEM Single Cell Multiome ATAC + GEX libraries recapitulate data obtained with the standalone Chromium Next GEM Single Cell ATAC v1.1 and Chromium Next GEM Single Cell 3' GEX v3.1 assays.

Table 1. Sequencing sensitivity, gene expression profiling, and cell profiling are highly consistent between Chromium Next GEM Single Cell Multiome Gene Expression libraries and Chromium Next GEM Single Cell 3' Gene Expression v3.1 libraries.

Single Cell Multiome Gene Expression vs Single Cell 3' Gene Expression v3.1
Sensitivity and Sequencing Saturation

To compare sensitivity, median genes and UMIs are plotted against sequenced raw read pairs. Median genes and UMIs captured from the Single Cell Multiome ATAC + GEX assay are comparable to those obtained by the standalone Single Cell 3' GEX v3.1. At 20,000 read pairs per cell, both assays reach ~50% sequencing saturation. Introns reads were mapped for both assays as nuclei are expected to have a greater proportion of intronic reads.



Gene Expression Profile: Assay-to-assay and replicate-to-replicate consistency

Technical replicates for each assay were aggregated and normalized by sequencing depth. The single cell barcode t-SNE plots show the gene expression data analyzed and visualized using Cell Ranger aggr and Loupe Cell Browser, respectively. The tSNE plots of the Single Cell Multiome GEX and Single Cell 3' GEX v3.1 libraries show significant overlap as evidenced by the consistent cluster structure across both assays and both replicates.

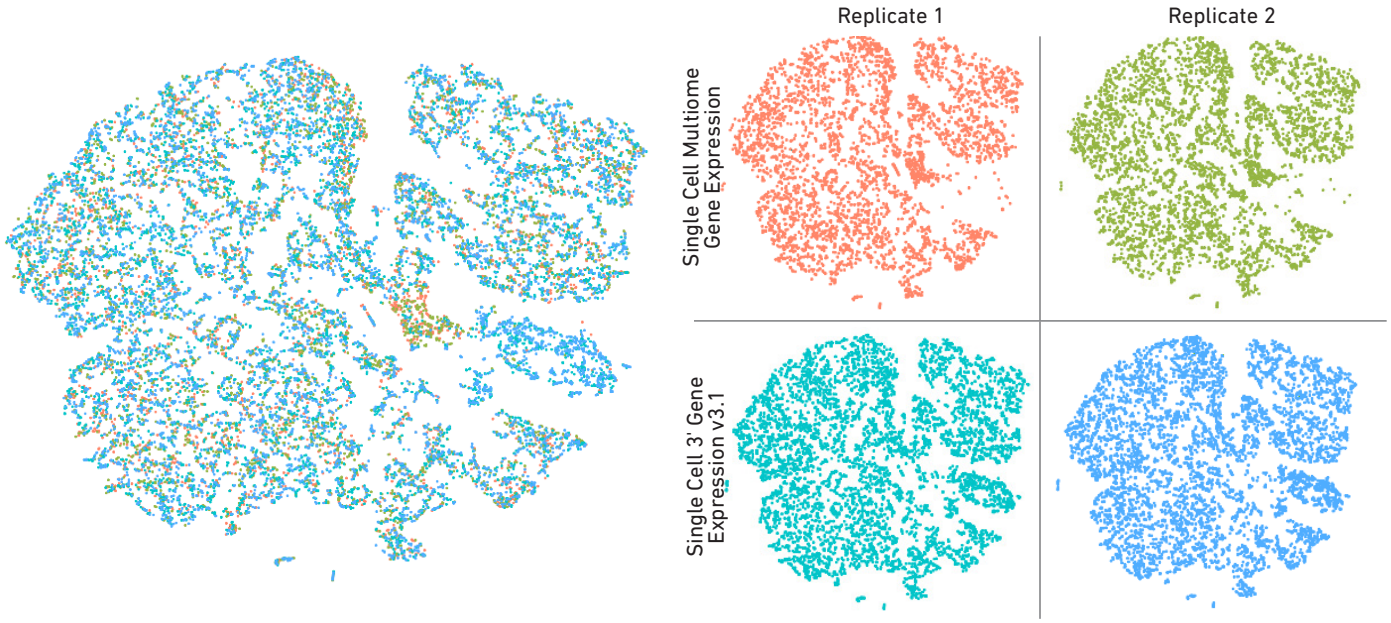
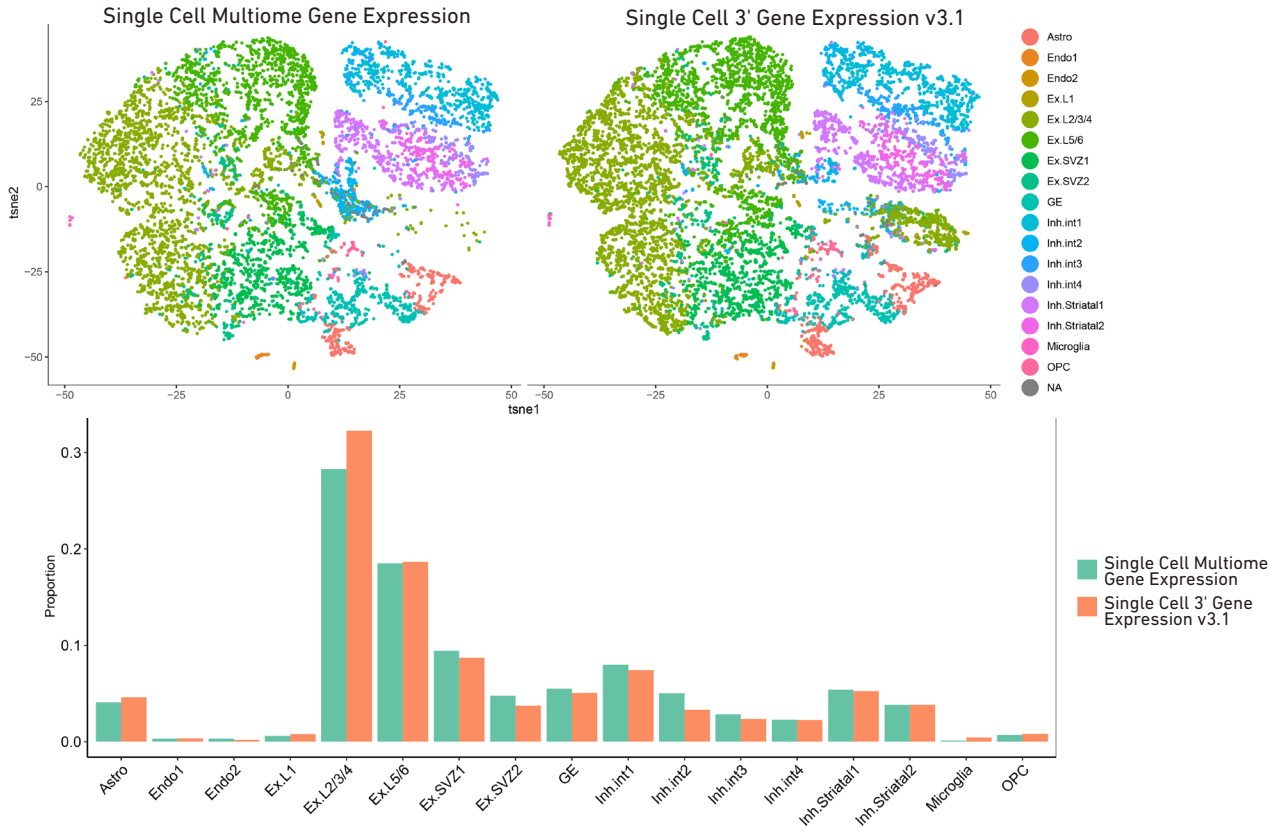


Table 1 contd.

Single Cell Multiome Gene Expression vs Single Cell 3' Gene Expression v3.1

Conserved Cell Type Proportions

Major cell populations in each cluster were identified via known marker genes for Single Cell Multiome GEX and Single Cell 3' GEX v3.1 libraries. Comparable cell type proportions among the two sets of data are also shown in the graph below, demonstrating that the Multiome GEX workflow conserves the cell type profiling observed with Single Cell 3' GEX v3.1.



Astro: Astrocytes
Endo: Endothelial cells
Ex: Excitatory neurons
GE: Ganglionic eminence
Inh: Inhibitory neurons
Int: Interneurons
OPC: Oligodendrocyte progenitor cells
SVZ: Subventricular zone

Conserved Cell Type Specific Markers

To investigate possible changes in gene expression due to the assay workflow, expression of major neuronal cell type markers were compared in data generated using either the Single Cell Multiome GEX or Single Cell 3'GEX v3.1 workflows. All the key markers were detected in both datasets at comparable amounts.

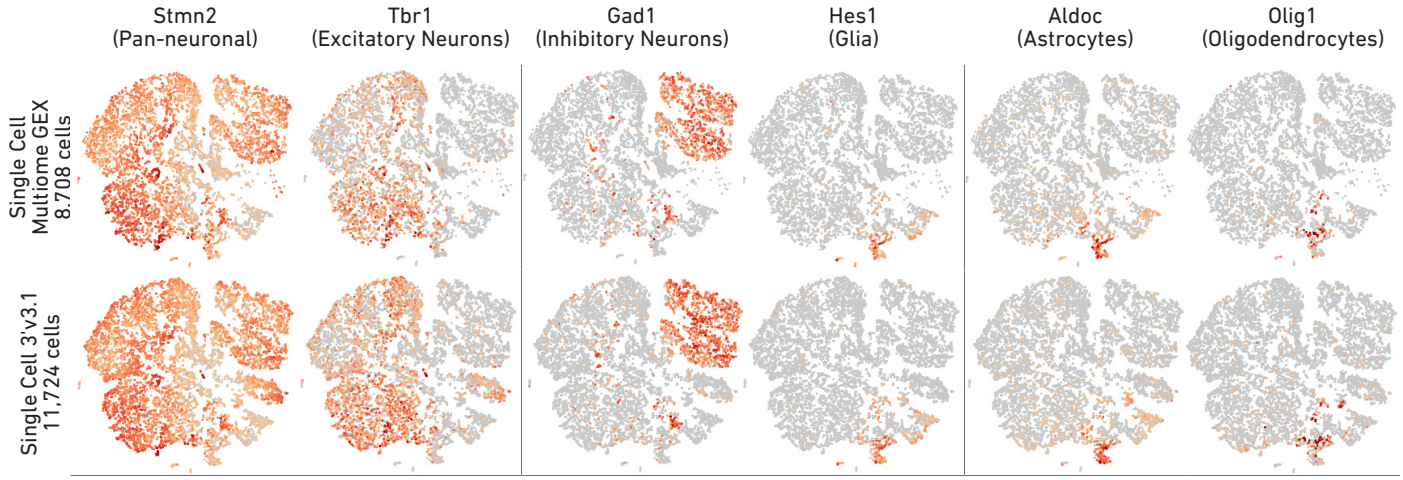


Table 1 contd.

Conserved Cell Type Specific Markers
Comparison of all genes expressed in the Multiome GEX libraries were highly correlated with genes expressed in Single Cell 3' Gene Expression v3.1 libraries with a pearson correlation value of 0.89.

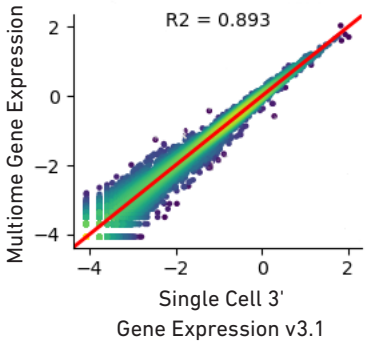
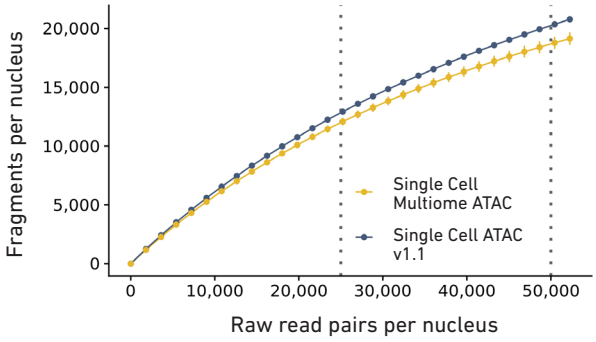


Table 2. Sequencing sensitivity, chromatin profiling, cell profiling, and chromatin accessible fragments are highly consistent between Chromium Next GEM Single Cell Multiome ATAC libraries and Chromium Next GEM Single Cell ATAC v1.1 libraries.

Single Cell Multiome ATAC vs Single Cell ATAC v1.1
Sensitivity and Sequencing Saturation
To compare ATAC sensitivity, sequenced fragments are plotted against sequenced raw read pairs. The number of ATAC fragments captured by the Single Cell Multiome ATAC + GEX solution is comparable to those obtained by the standalone Single Cell ATAC v1.1 solution.



Accessible Chromatin Profile: Assay-to-assay and replicate-to-replicate consistency
Technical replicates for each assay were aggregated and normalized by sequencing depth using custom scripts. The resulting tSNE plots for each assay and replicates are shown below. The tSNE plots of the Single Cell Multiome ATAC and Single Cell ATAC v1.1 libraries show significant overlap as the cluster structure remains consistent across both assays and across both replicates.

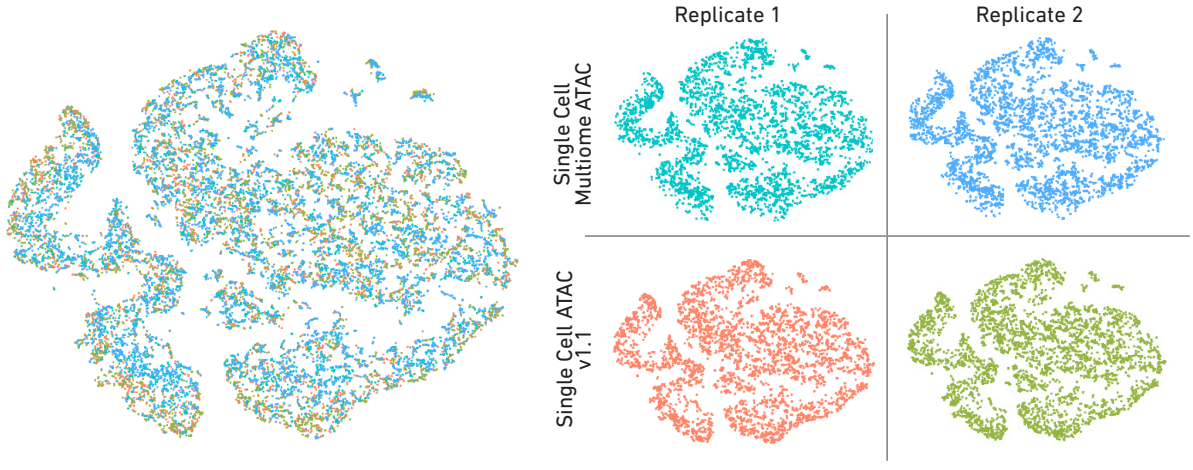
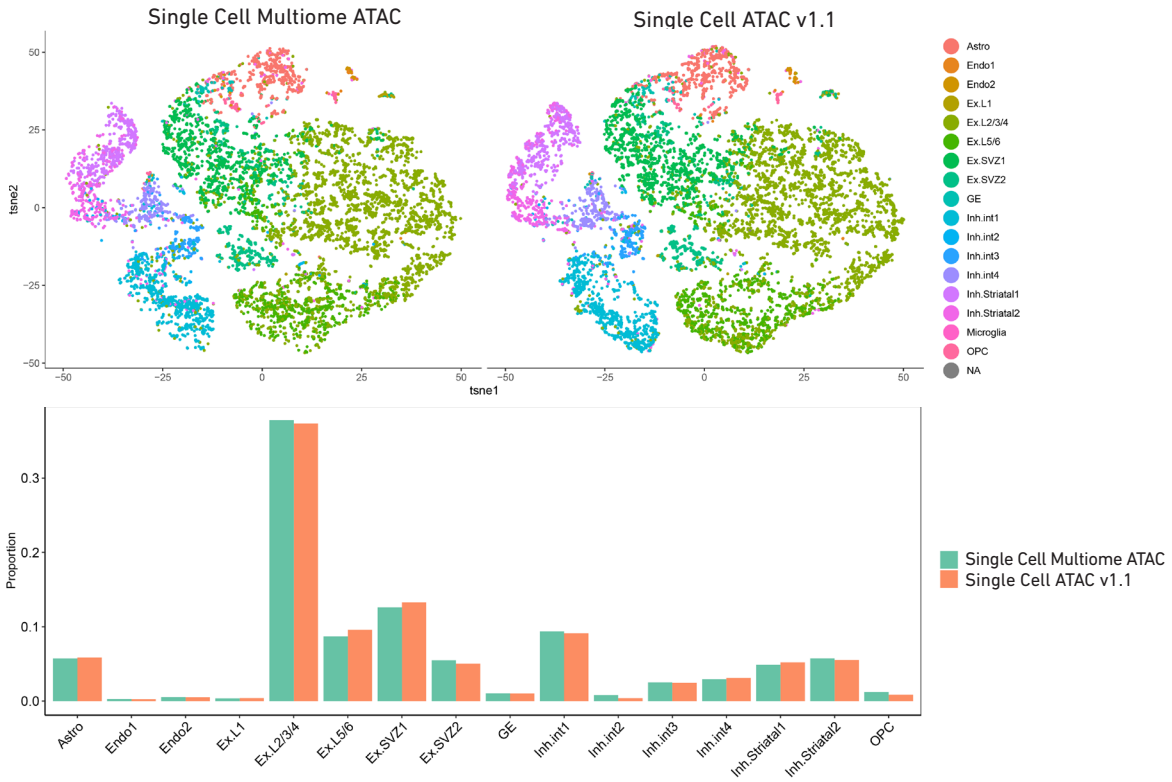


Table 2 contd.

Single Cell Multiome ATAC vs Single Cell ATAC v1.1

Conserved Cell Type Proportions

Major cell populations in each cluster were identified via known marker genes for Single Cell Multiome ATAC and Single Cell ATAC v1.1 libraries. Comparable cell type proportions among the two sets of data are also shown in the graph below, demonstrating that the Single Cell Multiome ATAC workflow conserves the cell type profiling observed with Single Cell ATAC v1.1.



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Conserved Cell Marker Promotor Accessibility

To investigate possible changes in chromatin accessibility due to the assay workflow, the accessibility of known neuronal cell markers was compared in data generated using either the Single Cell Multiome ATAC or Single Cell ATAC v1.1 workflows. All the markers assessed showed similar accessibility profiles across both assays.

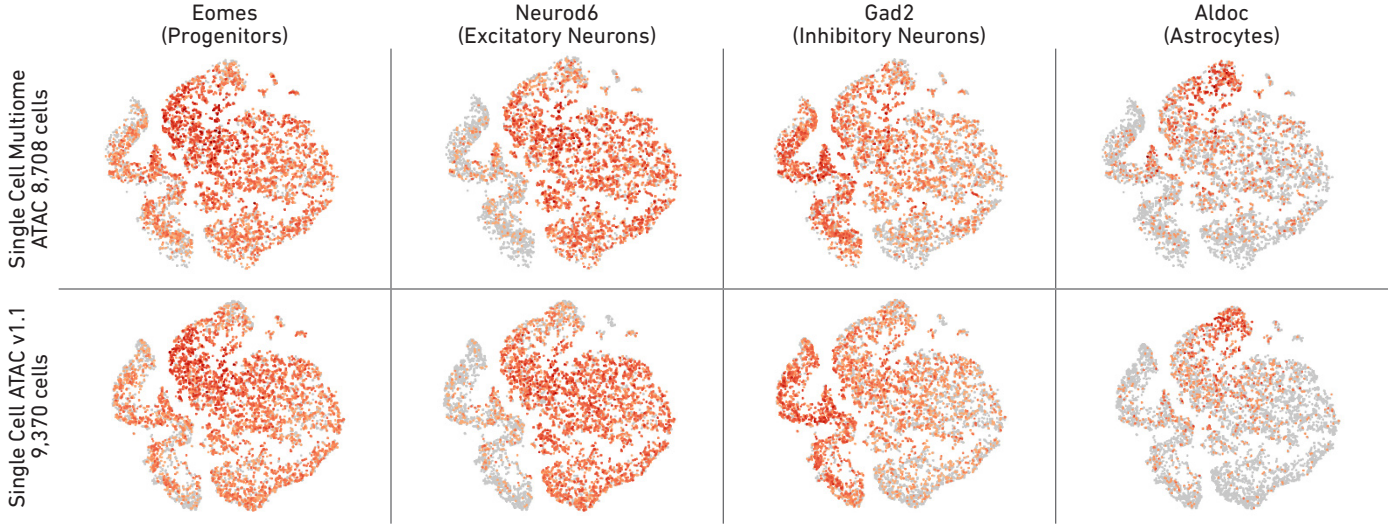
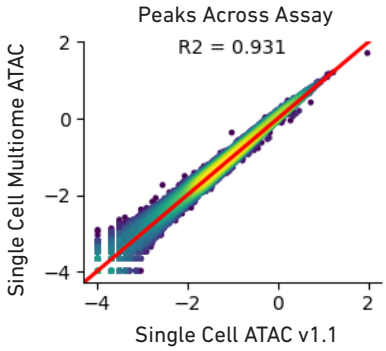


Table 2 contd.

Single Cell Multiome ATAC vs Single Cell ATAC v1.1
Conserved Cell Type Specific Markers

Comparison of all peaks called in the Single Cell Multiome ATAC libraries were highly correlated with peaks called in Single Cell ATAC v1.1 libraries with a pearson correlation value of 0.931.



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