Chromium X Series: Single Cell Data Highlights and Comparisons

Introduction

The Chromium X Series offers a highly optimized approach to single cell studies and enables both low and high throughput experiments. All 10x Genomics single cell dual index assays are supported on Chromium X Series instruments. This Technical Note compares data generated from Chromium Next GEM Single Cell 3', 5', ATAC, and Multiome ATAC + Gene Expression assays run on both the Chromium Controller and Chromium X.

The Chromium X Series is the next generation of 10x Genomics instrumentation, purposefully designed to enable high-throughput experiments. Compared to the Chromium Controller, the Chromium X Series has:

- Additional sensors and more accurate pressure and tray temperature control.
- A highly interactive user interface with familiar one-button operation.
- Seamless firmware upgrades, network connectivity, and syncing to the 10x Genomics Cloud, to log run information and experimental setups.
- Similar performance as assessed by multiplet rates, library complexity, read mapping rates, and cell clustering.
- Ability to run an assay with low throughput (LT), standard, or high throughput (HT).
- Low throughput: 100–1,000 cells per channel
- Standard assay: 500–10,000 cells per channel
  500-30,000 cells per channel with CellPlex
- High throughput: 2,000–20,000 cells per channel
  2,000–60,000 cells per channel with CellPlex

Chromium Next GEM Assays	Chromium		
	Controller	iX	X
Single Cell 3' Gene Expression LT v3.1 (Dual Index)	✓	✓	✓
Single Cell 3' Gene Expression v3.1 (Dual Index)	✓	✓	✓
Single Cell 3' Gene Expression HT v3.1 (Dual Index)	No	Upgrade Required	✓
Single Cell 3' Gene Expression (Single Index)	✓	✓	✓
Single Cell Immune Profiling v2 (Dual Index)	✓	✓	✓
Single Cell Immune Profiling v2 HT (Dual Index)	No	Upgrade Required	✓
Single Cell Immune Profiling v1.1	✓	✓	✓
Single Cell ATAC v1.1	✓	✓	✓
Single Cell Multiome ATAC + Gene Expression	✓	✓	✓

Table 1. Assay and instrument compatibility. Check marks indicate that the indicated assay will run successfully on the indicated instrument.

The Chromium X Series is one instrument, available in two firmware options (Chromium X and Chromium iX). A comparison of Controller, X, and iX capabilities can be found in Table 1.

To compare library quality between the Chromium Controller and Chromium X, cells (293T, 3T3: ATCC, Peripheral Blood Mononuclear Cells (PBMCs): AllCells)) and nuclei (GM12878: Coriell Institute; EL4: ATCC; PBMCs: AllCells) were prepared according to the Demonstrated Protocols listed in the References section.

Samples were processed in parallel according to the following library construction workflows as shown in Figure 1:

- Chromium Next GEM Single Cell 3' Gene Expression v3.1 (standard and low throughput)
- Chromium Next GEM Single Cell Immune Profiling v2
- Chromium Next GEM Single Cell Immune Profiling v1.1
- Chromium Next GEM Single Cell ATAC v1.1
- Chromium Next GEM Single Cell Multiome ATAC + Gene Expression.

The User Guides used for library construction are listed in the References section. High throughput samples were not analyzed for this Technical Note. Data from high throughput samples may be found in the Technical Notes listed in the References section.

After loading the appropriate chip, samples were run on either the Chromium Controller or Chromium X to generate Gel Beads-in-emulsion. After cleanup and cDNA amplification, single cell libraries were constructed according to the User Guides above. The libraries were sequenced and the data were analyzed using Cell Ranger 6.1 for Single Cell 3' and Immune Profiling libraries, Cell Ranger ATAC 2.0 for Single Cell ATAC libraries, and Cell Ranger ARC 2.0 for Single Cell Multiome ATAC + Gene Expression libraries.

Figures 2-13 demonstrate that the single cell data generated from samples run on the Chromium Controller and Chromium X have comparable multiplet rates and produce libraries with similar complexity, read mapping, and cell clustering.

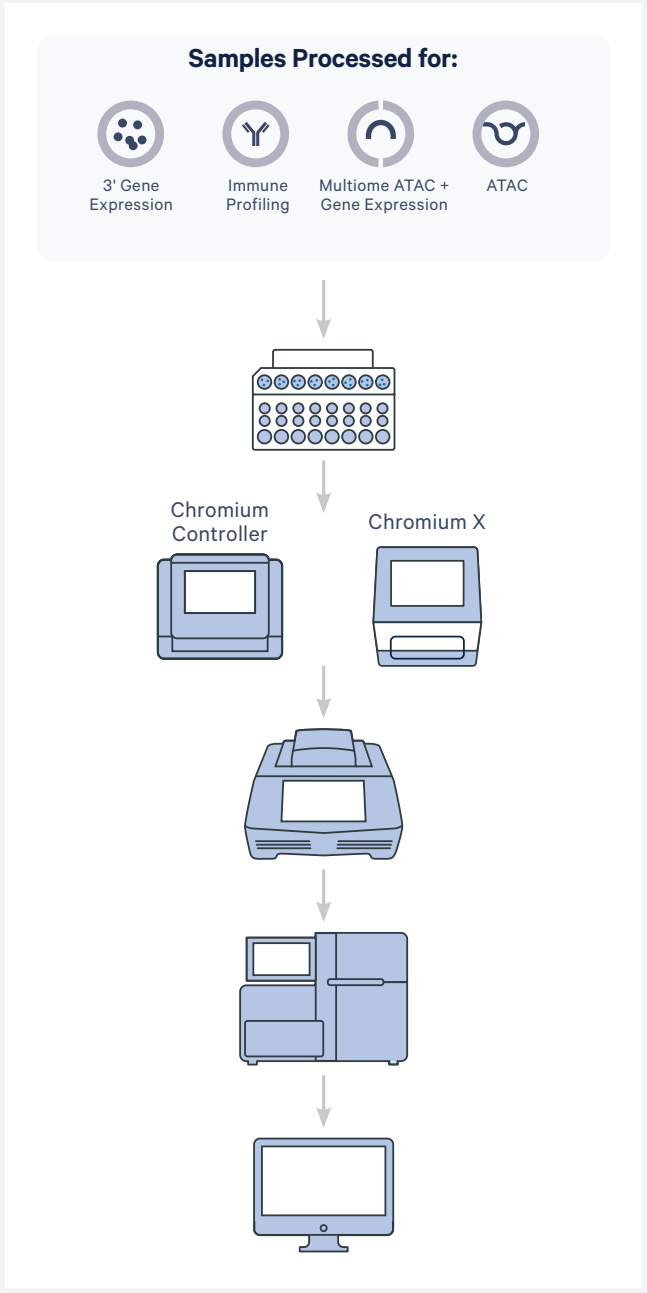


Figure 1. Samples were prepared for four different single cell workflows, run on both the Chromium Controller and Chromium X.

3' Single Cell Gene Expression

Cell Multiplet Rates, Library Complexity, and Read Mapping

Libraries in the following figures were constructed from human 293T and mouse 3T3 cells (ATCC). Cells were loaded onto two Single Cell 3' v3.1 Next GEM Chip Gs (10,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

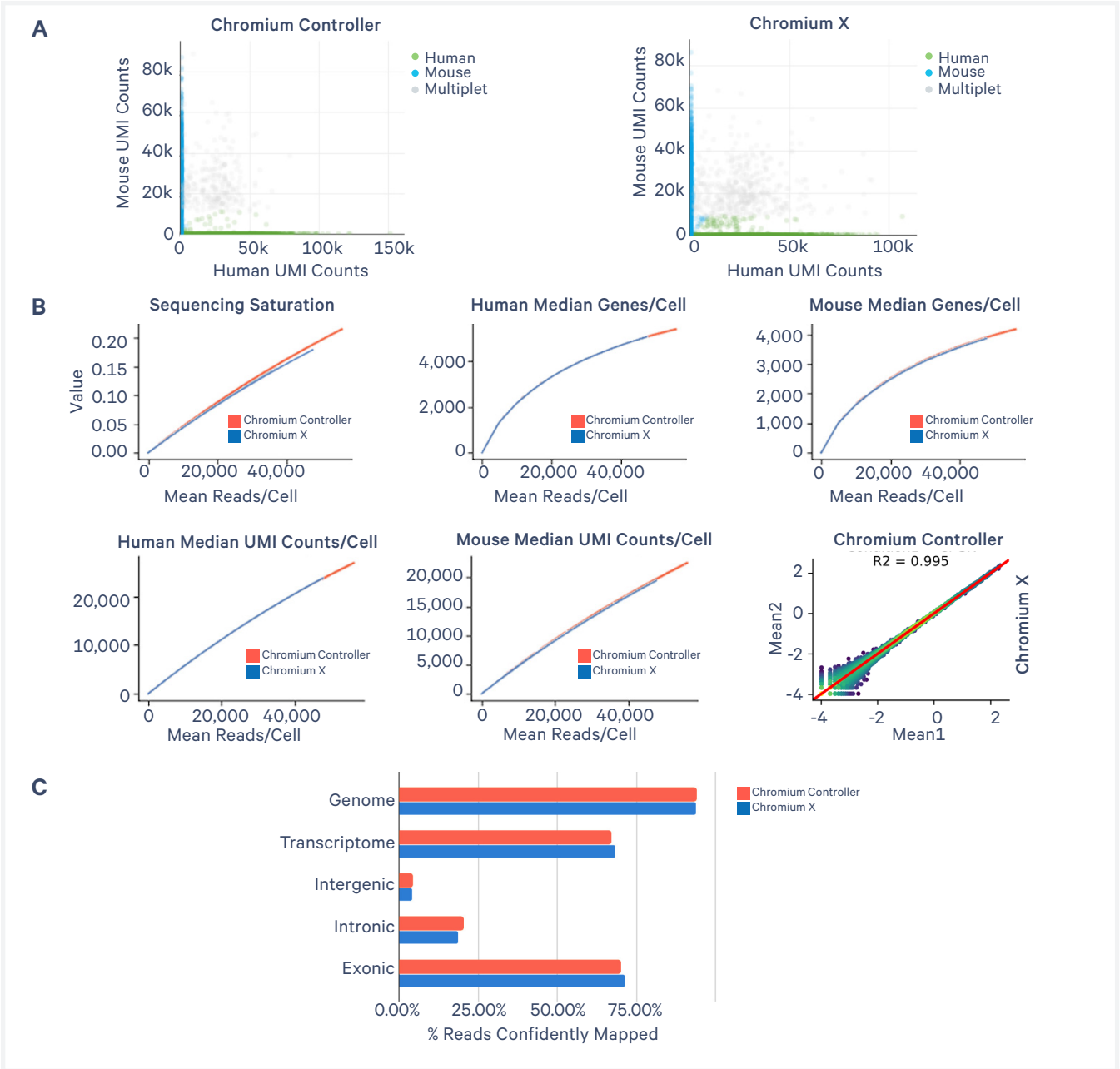


Figure 2. Libraries generated using the Chromium Next GEM Single Cell 3' v3.1 (Dual Index) Gene Expression kits in parallel on both the Chromium Controller and Chromium X show comparable multiplet rates of ~1.0% per 1,000 cells recovered (A). Comparable library complexity and chemistry correlation was observed in Single Cell 3' v3.1 Gene Expression data between the Chromium Controller and Chromium X as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation (B). Comparable mapping rates between the two instruments are shown in (C).

3' Single Cell Gene Expression

Library Complexity, Read Mapping, and Cell Clustering

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were loaded onto two Single Cell 3' v3.1 Next GEM Chip Gs (10,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

Figure 3. Chromium Next GEM Single Cell 3' v3.1 Gene Expression run on both the Chromium Controller and Chromium X show comparable library complexity and chemistry correlation as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation. Comparable mapping rates between the two instruments are shown in (B). Similar cellular populations were detected in PBMCs profiled using the Single Cell 3' v3.1 on either Chromium Controller or Chromium X (C). Aggregated t-SNE plots from both assays (left), t-SNE plot from the Chromium Controller (top right), and t-SNE plot from the Chromium X (bottom right) show comparable cell subpopulations based on gene expression data.

5'v2 Single Cell Gene Expression

Cell Multiplet Rates, Library Complexity, and Read Mapping

Libraries in the following figures were constructed from human 293T and mouse 3T3 cells (ATCC). Cells were loaded onto two Single Cell 5' v2 Next GEM Chip Ks (10,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

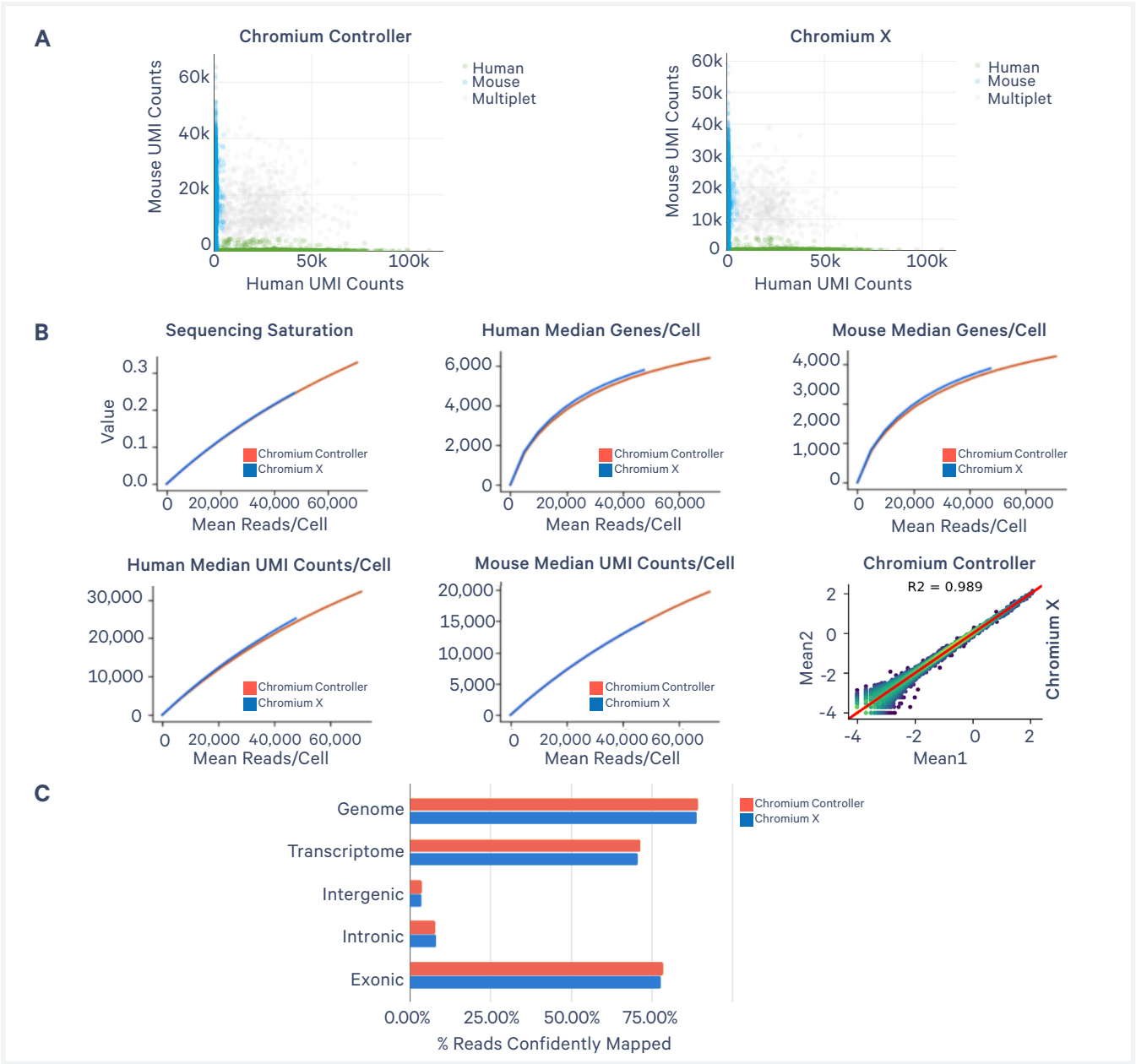


Figure 4. Chromium Next GEM Single Cell 5' v2 Gene Expression run on both the Chromium Controller and Chromium X show comparable multiplet rates of ~1.0% (A). Comparable library complexity and chemistry correlation was observed in Single Cell 5' v2 data between the Chromium Controller and Chromium X as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation (B). Comparable mapping rates between the two instruments are shown in (C).

5'v2 Single Cell Gene Expression

Library Complexity, Read Mapping, and Cell Clustering

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were loaded onto two Single Cell 5' v2 Next GEM Chip Ks (10,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

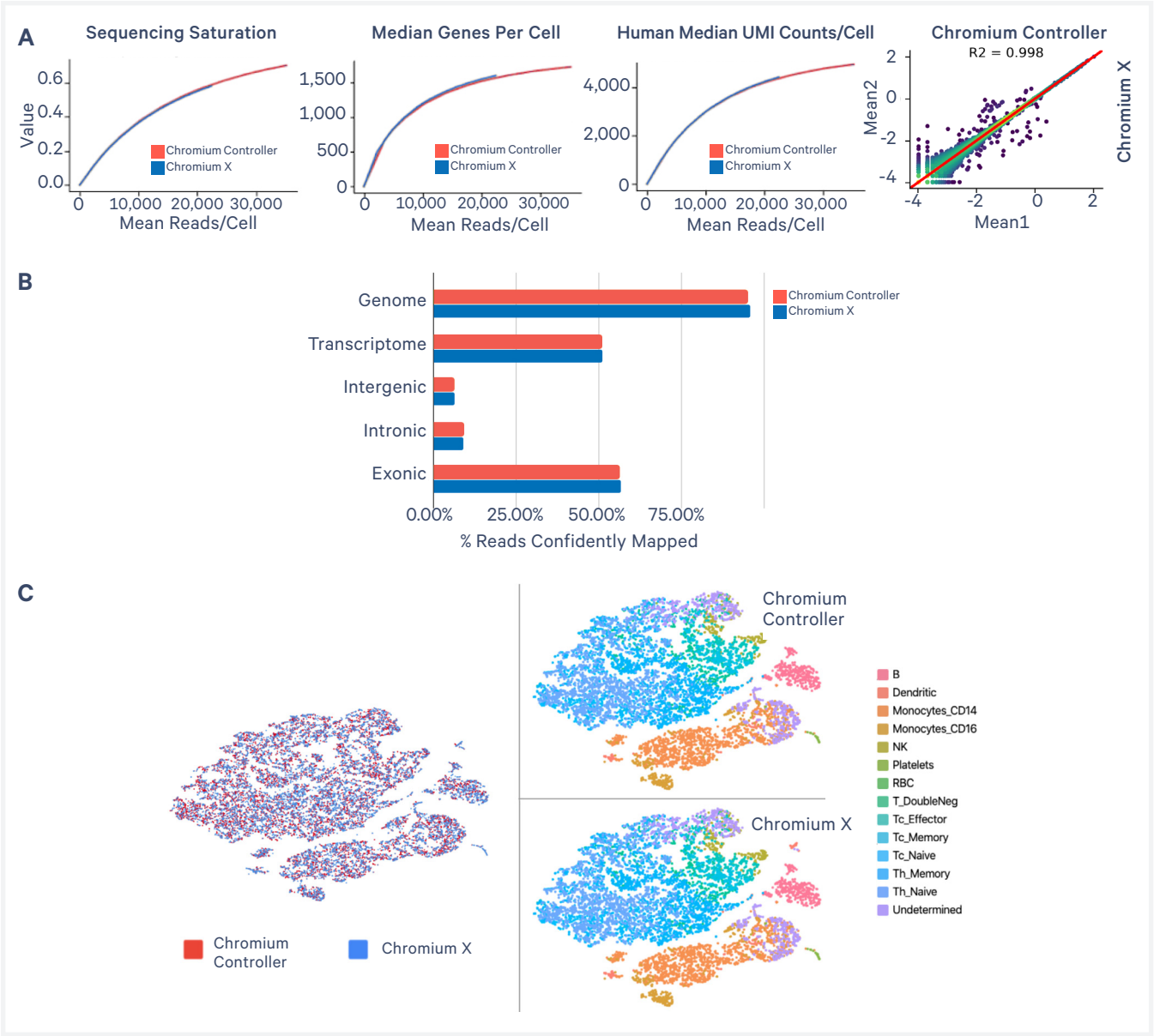
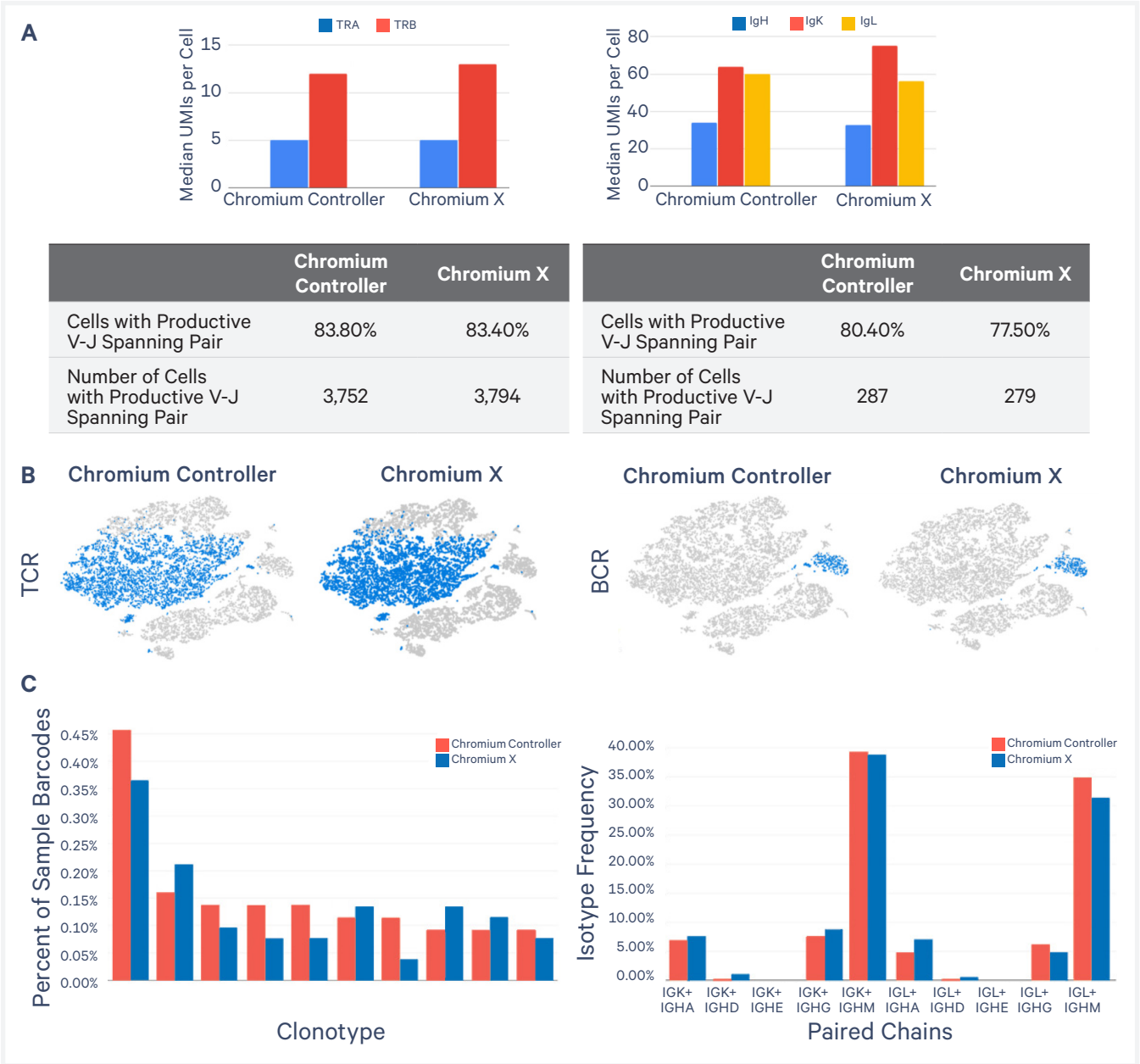


Figure 5. Chromium Next GEM Single Cell 5' v2 Gene Expression run on both the Chromium Controller and Chromium X show comparable library complexity and chemistry correlation as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation. Comparable mapping rates between the two instruments are shown in (B). Similar cellular populations were detected in PBMCs profiled using the Single Cell 5' v2 on Chromium Controller or Chromium X (C). Aggregated t-SNE plots from both assays (left), t-SNE plot from the Chromium Controller (top right), and t-SNE plot from the Chromium X (bottom right) show comparable cell subpopulations based on gene expression data.

V(D)J v2

V(D)J Clonotypes

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were loaded onto two Single Cell 5' v2 Next GEM Chip Ks (10,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.



	Chromium Controller	Chromium X
Cells with Productive V-J Spanning Pair	83.80%	83.40%
Number of Cells with Productive V-J Spanning Pair	3,752	3,794

	Chromium Controller	Chromium X
Cells with Productive V-J Spanning Pair	80.40%	77.50%
Number of Cells with Productive V-J Spanning Pair	287	279

Figure 6. Similar TRA/TRB and IgH/IgK/IgL UMIs were detected in PBMCs profiled using the Single Cell 5' v2 on Chromium Controller or Chromium X (A). Samples also exhibit similar cells with productive spanning pairs between instruments. V(D)J clonotypes are detected in the same population of cells in PBMCs profiled using the Single Cell 5' v2 on Chromium Controller or Chromium X (B). Top TCR clonotypes and BCR paired chain usage is consistent across samples (C).

5'v1.1 Single Cell Gene Expression

Library Complexity, Read Mapping, and Cell Clustering

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were loaded onto two Single Cell 5' v1.1 Next GEM Chip Gs (2,000 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

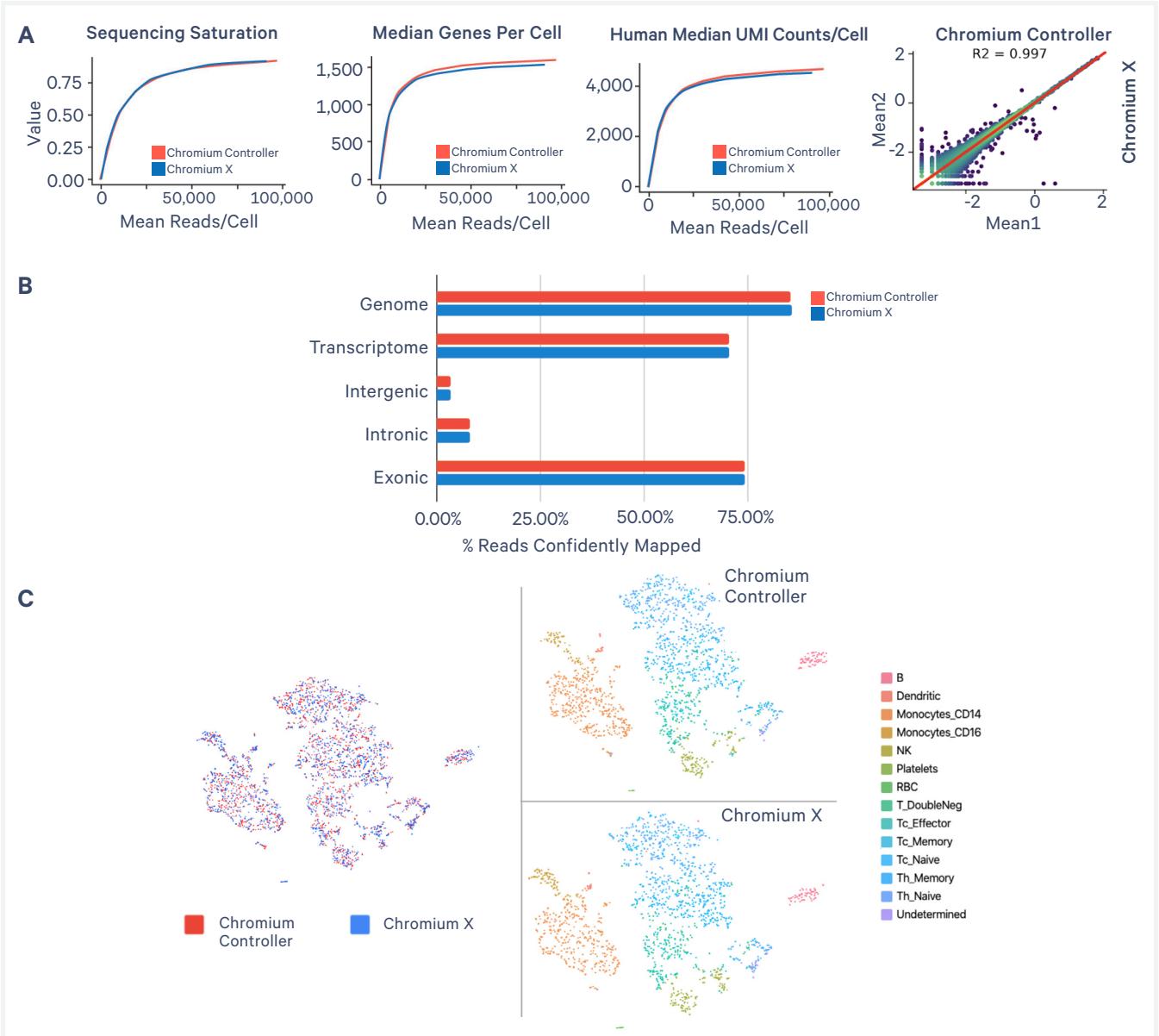


Figure 7. Chromium Next GEM Single Cell 5' v1.1 Gene Expression run on both the Chromium Controller and Chromium X show comparable library complexity and chemistry correlation as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation. Comparable mapping rates between the two instruments are shown in (B). Similar cellular populations were detected in PBMCs profiled using the Single Cell 5' v1.1 on Chromium Controller or Chromium X (C). Aggregated t-SNE plots from both assays (left), t-SNE plot from the Chromium Controller (top right), and t-SNE plot from the Chromium X (bottom right) show comparable cell subpopulations based on gene expression data.

Single Cell ATAC

Cell Multiplets, Library Complexity, and Read Mapping

Libraries in the following figures were constructed from human GM12878 (Coriell Institute) and EL4 cells (ATCC). Cells were thawed and lysed according to the Nuclei Isolation for Single Cell ATAC Sequencing Demonstrated Protocol (CG000169). Nuclei were loaded onto two Single Cell ATAC v1.1 Next GEM Chip Hs (10,000 nuclei targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

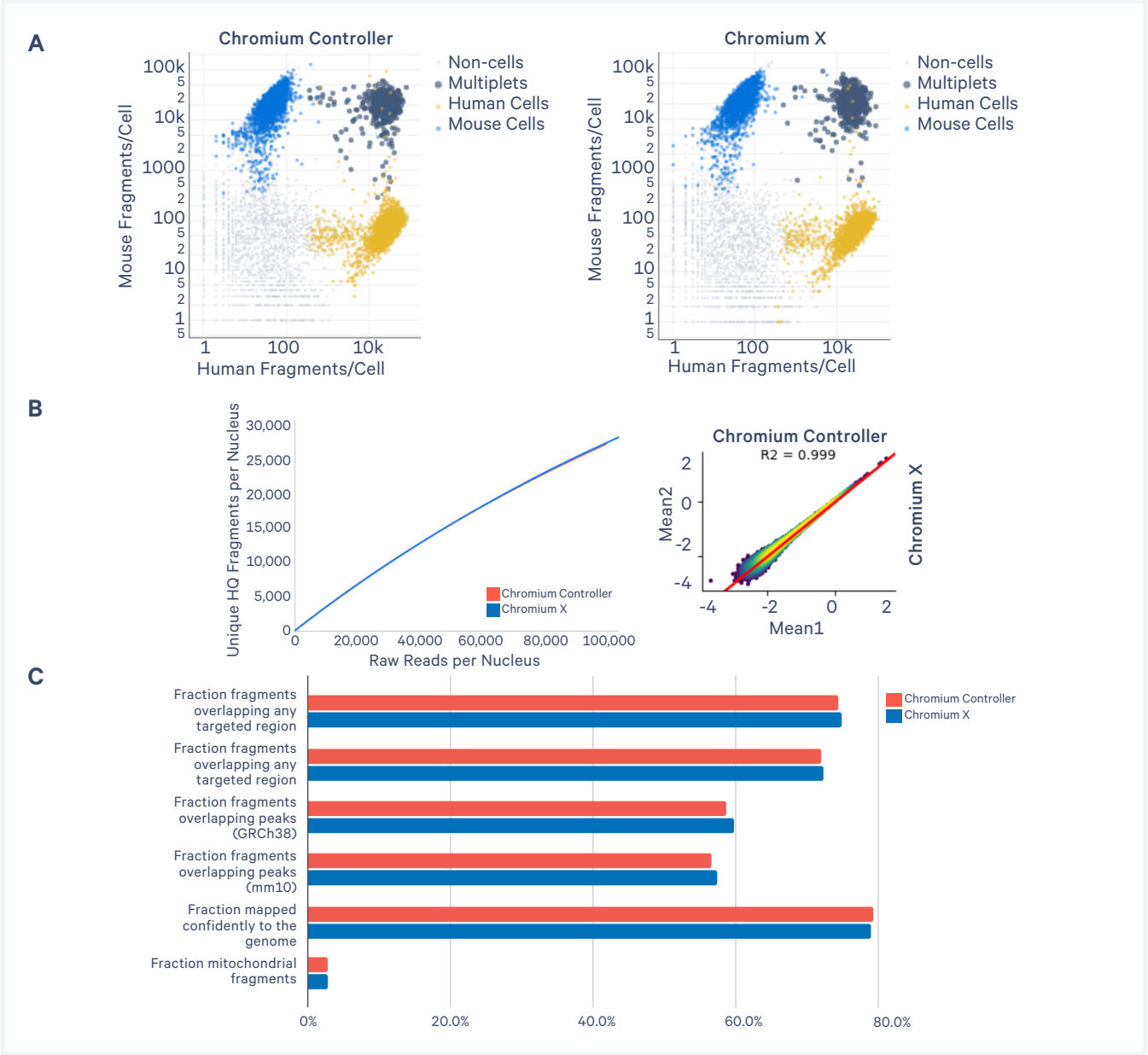


Figure 8. Low multiplet rates are observed for Single Cell ATAC v1.1 samples run on Chromium Controller vs Chromium X(A). Number of fragments mapping to the human or mouse genome for each 10x barcode. 10x barcode not detected as cells are light grey. Cells with fragments mapping to human (yellow), mouse (blue), or both (dark gray, multiplets) are shown for Chromium Controller and Chromium X (~1.0% multiplets per 1k nuclei). Comparable unique fragments per nucleus and peak correlation plots were derived from the two instruments are shown in (B). Comparable read mapping rates are shown in (C)

Single Cell ATAC

Library Complexity, Read Mapping, and Cell Clustering

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were thawed and lysed according to the Nuclei Isolation for Single Cell ATAC Sequencing Demonstrated Protocol (CG000169). Nuclei were loaded onto two Single Cell ATAC v1.1 Next GEM Chip Hs (10,000 nuclei targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

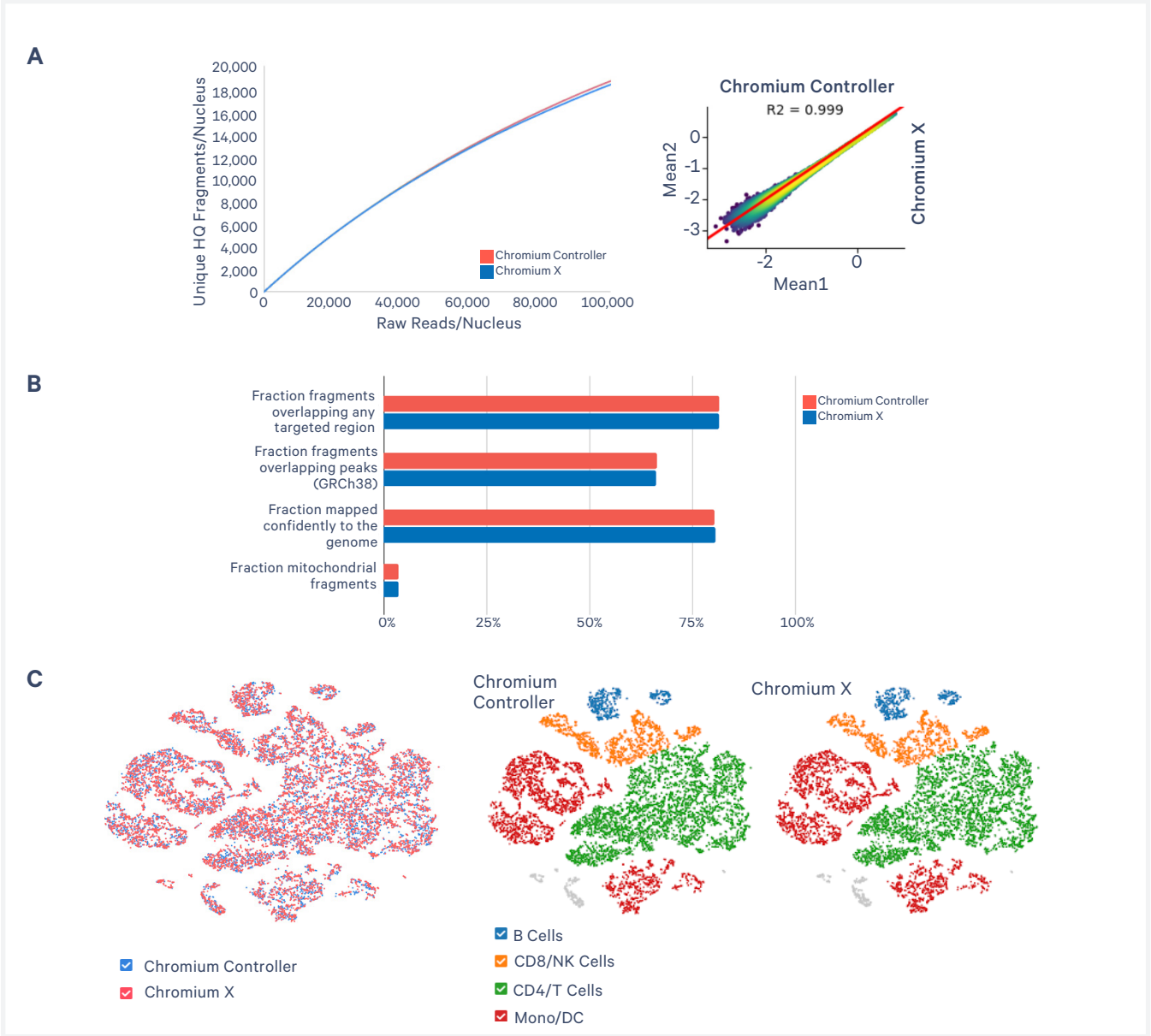


Figure 9. Comparable library complexity and peak correlation was observed in the Single Cell ATAC v1.1 on Chromium Controller vs Chromium X (A). Comparable unique fragments per nucleus (left) and peak correlation (right) plots were derived from the two instruments. Comparable read mapping rates are shown in (B). Similar cellular populations were detected in PBMCs profiled using the Single Cell ATAC v1.1 on Chromium Controller vs Chromium X (C). Aggregated t-SNE plots from both instruments (left). t-SNE plot from the Chromium Controller and Chromium X (right) show comparable cell subpopulations based on ATAC.

Single Cell Multiome ATAC + Gene Expression

Library Complexity and Read Mapping

Libraries in the following figures were constructed from PBMCs (AllCells). Cells were thawed and lysed according to the Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000365). Nuclei were loaded onto two Single Cell Multiome Next GEM Chip Js (10,000 nuclei targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

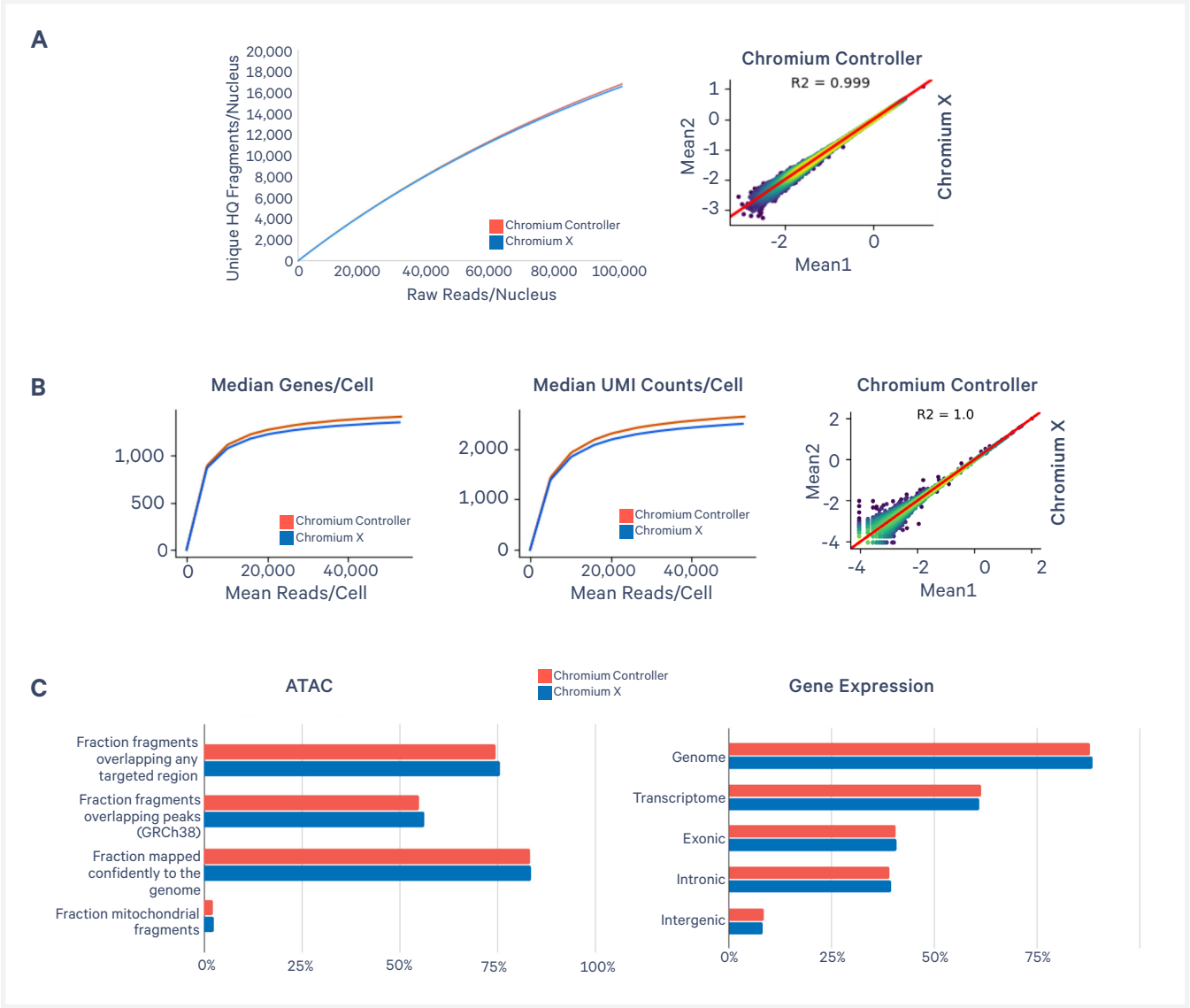


Figure 10. Comparable library complexity and peak correlation was observed in the Single Cell Multiome on Chromium Controller vs Chromium X (A). Comparable unique fragments per nucleus (left) and peak correlation plots (right) were derived from the two instruments. Comparable Gene Expression library complexity was observed in Single Cell Multiome on Chromium Controller vs Chromium X (B). Comparable median genes per cell, UMIs per cell, and UMI correlation plots were derived from the two instruments. Comparable read mapping rates for are shown in (C) for ATAC (left) and Gene Expression (right). Cell multiplet rates are not shown, but are within expected ranges.

Single Cell Multiome

Cell Clustering

Libraries in the following figures were constructed from PBMCs. Libraries in the following figures were constructed from PBMCs (AllCells). Cells were thawed and lysed according to the Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000365). Nuclei were loaded onto two Single Cell Multiome Next GEM Chip Js (10,000 nuclei targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

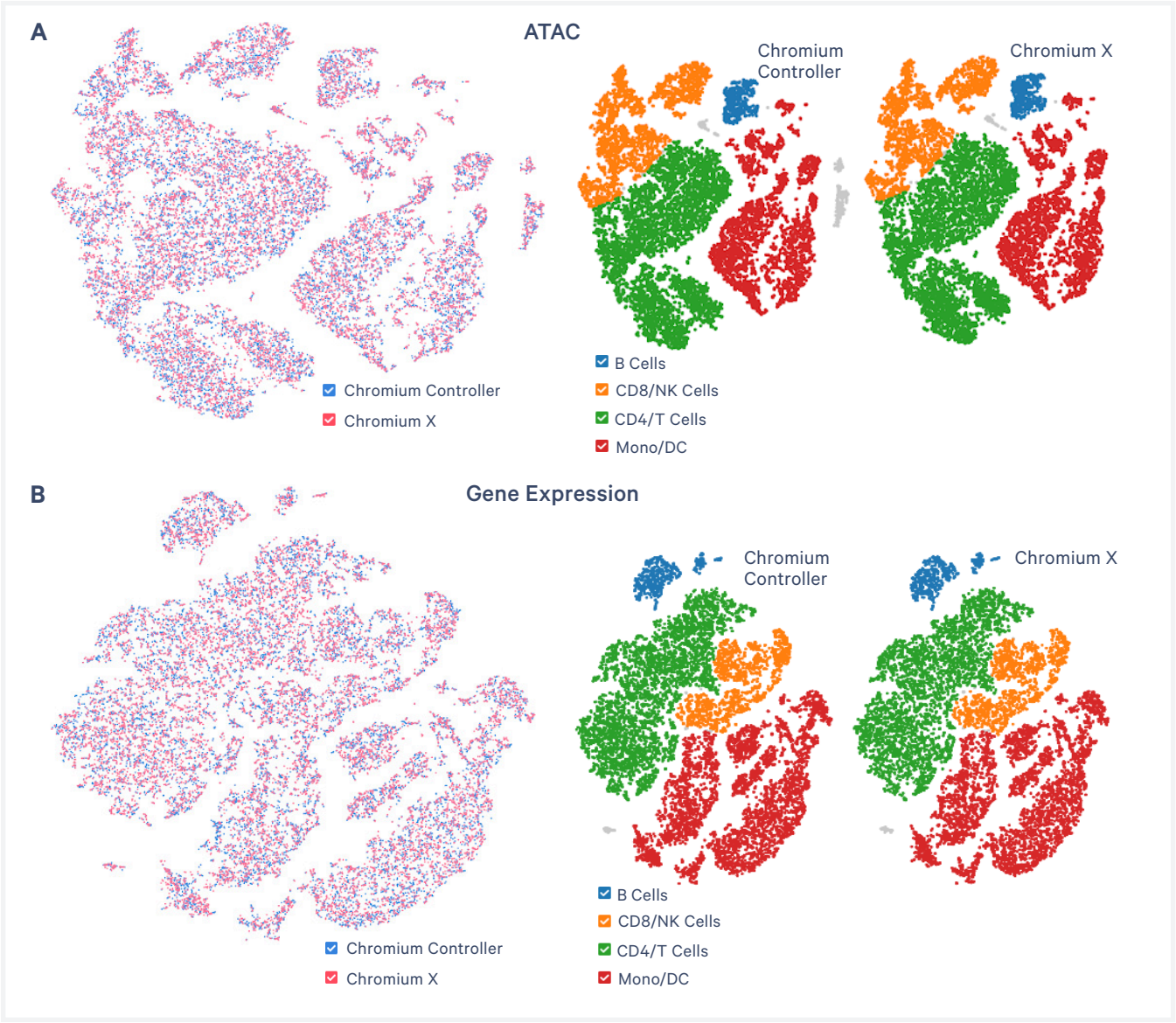


Figure 11. Similar cellular populations were detected in PBMCs profiled using Single Cell Multiome on Chromium Controller vs Chromium X and clustered using chromatin accessibility profiles (A). Aggregated t-SNE plots from both instruments (left). t-SNE plot from the Chromium Controller and Chromium X (right) show comparable cell subpopulations based on ATAC. Similar cellular populations were detected in PBMCs profiled using Single Cell Multiome on Chromium Controller vs Chromium X and clustered using gene expression (B). Aggregated t-SNE plots from both instruments (left). t-SNE plot from the Chromium Controller and Chromium X (right) show comparable cell subpopulations based on gene expression.

3' Single Cell Gene Expression - Low Throughput

Cell Multiplet Rates and Library Complexity

Libraries in the following figures were constructed from human 293T and mouse 3T3 cells (ATCC). Cells were loaded onto two Single Cell 3' LT v3.1 Next GEM Chip Ls (500 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

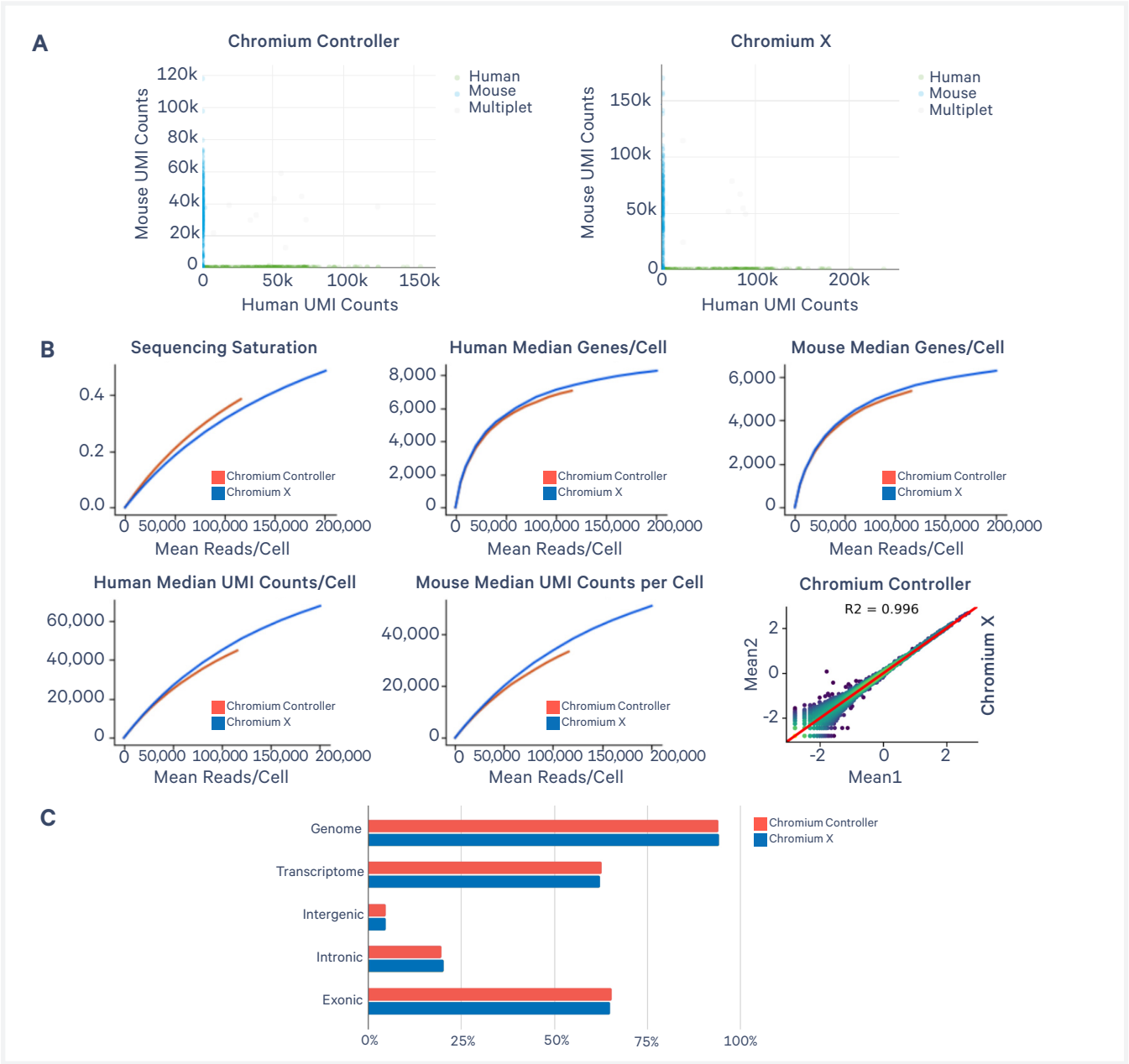


Figure 12. Libraries generated using the Chromium Next GEM Single Cell 3' LT v3.1 kit in parallel on both the Chromium Controller and Chromium X show comparable multiplet rates of 5.4% and 7.5% respectively (A). Comparable library complexity and chemistry correlation was observed in Single Cell 3' v3.1 Gene Expression - Low Throughput data between the Chromium Controller and Chromium X as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation (B). Comparable mapping rates between the two instruments are shown in (C).

3' Single Cell Gene Expression - Low Throughput

Library Complexity and Read Mapping

Libraries in the following figures were constructed from PBMCs. Cells were loaded onto two Single Cell 3' LT v3.1 Next GEM Chip Ls (500 cells targeted per replicate) and processed on Chromium Controller and Chromium X in parallel followed by library preparation, sequencing, and data analysis.

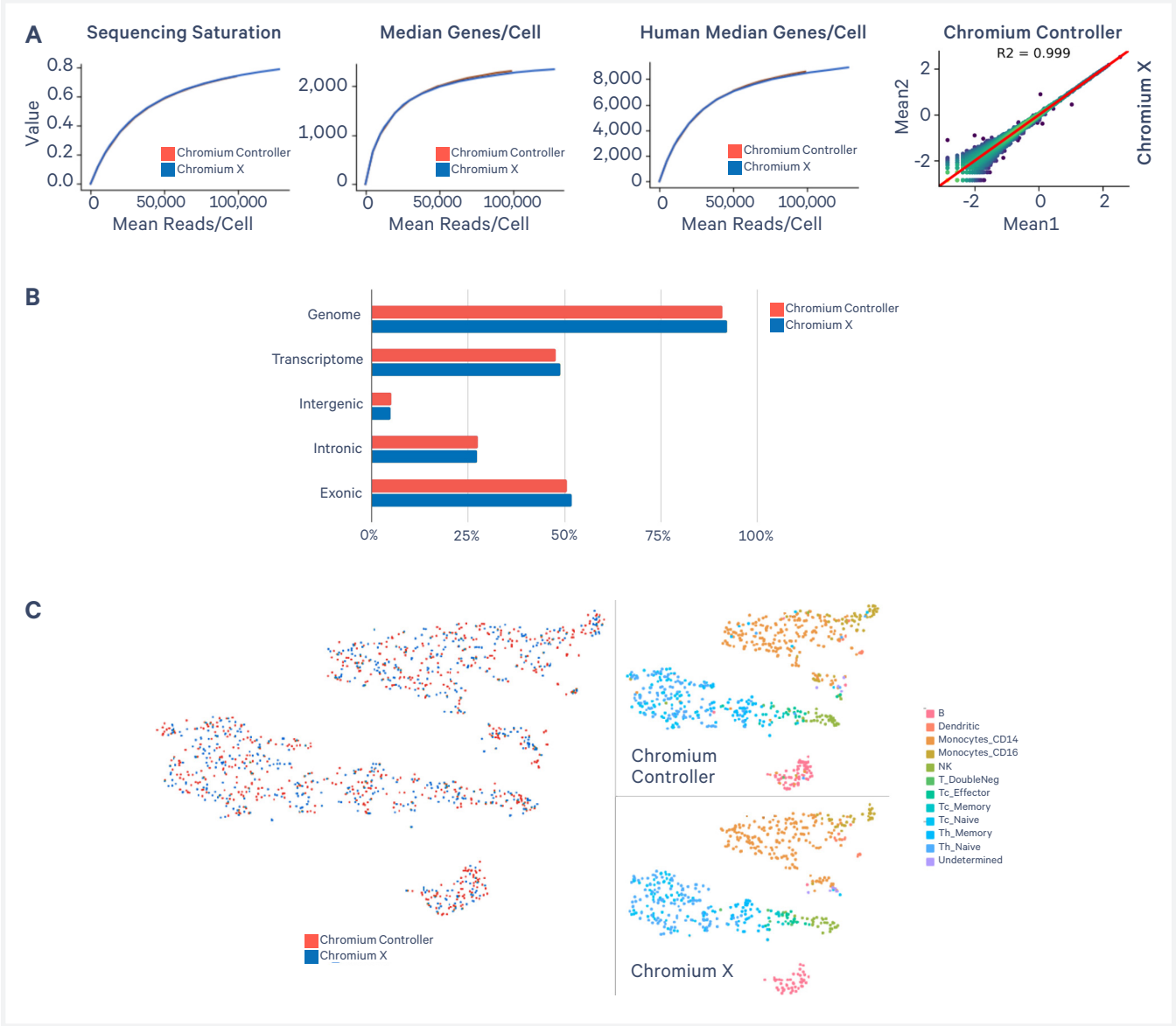


Figure 13. Chromium Next GEM Single Cell 3' LT v3.1 Gene Expression run on both the Chromium Controller and Chromium X show comparable library complexity and chemistry correlation as measured by sequencing saturation, median genes per cell, median UMI counts per cell, and UMI correlation. Comparable mapping rates between the two instruments are shown in (B). Similar cellular populations were detected in PBMCs profiled using the Single Cell 3' LT v3.1 on either Chromium Controller or Chromium X (C). Aggregated t-SNE plots from both instruments (left). t-SNE plot from the Chromium Controller and Chromium X (right) show comparable cell subpopulations based on gene expression.

Conclusion

Single cell assays performed in parallel on the Chromium Controller and the Chromium X show similar performance as assessed by multiplet rates, library complexity, read mapping rates, and cell clustering.

References

1. Nuclei Isolation for Single Cell ATAC Sequencing Demonstrated Protocol (CG000169)
2. Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000365)
3. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide (CG000315)
4. Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) User Guide (CG000399)
5. Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) User Guide (CG000331)
6. Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 User Guide (CG000207)
7. Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide (CG000209)
8. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338)
9. Chromium Next GEM Single Cell 3' HT v3.1: Reagents, Workflow & Data Overview (CG000422)
10. Chromium Next GEM Single Cell 5' HT v2: Reagents, Workflow & Data Overview (CG000425)

Document Revision Summary

Document Number	CG000428
Title	Chromium X Series: Single Cell Data Highlights and Comparison
Revision	Rev A to Rev B
Revision Date	November 2021

Specific Changes:

- Added Single Cell Immune Profiling v1.1 data
- Added CG000207, CG000422 and CG000425 to References

Specific Changes:

- Updated for general minor consistency of language and terms throughout

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