Chromium Connect Automated Single Cell 5' v2 with Feature Barcode technology: Workflow and Data Overview

Introduction

Chromium Next GEM Single Cell 5' v2 automated workflow with Feature Barcode technology is now available on the Chromium Connect Automated System. This automated workflow allows measuring cell surface protein expression and antigen specificity while simultaneously profiling single cell gene expression along with V(D)J immune repertoire mapping from the same pool of cells. This Technical Note shows comparable gene expression, immune repertoire, and cell surface protein expression data obtained using manual and automated Chromium Next GEM Single Cell 5' v2 with Feature Barcode technology workflow. In addition, the document shows cell surface protein expression data derived from automated single cell libraries generated from complex samples for immunophenotyping as well as for mapping antigen specific immune repertoire.

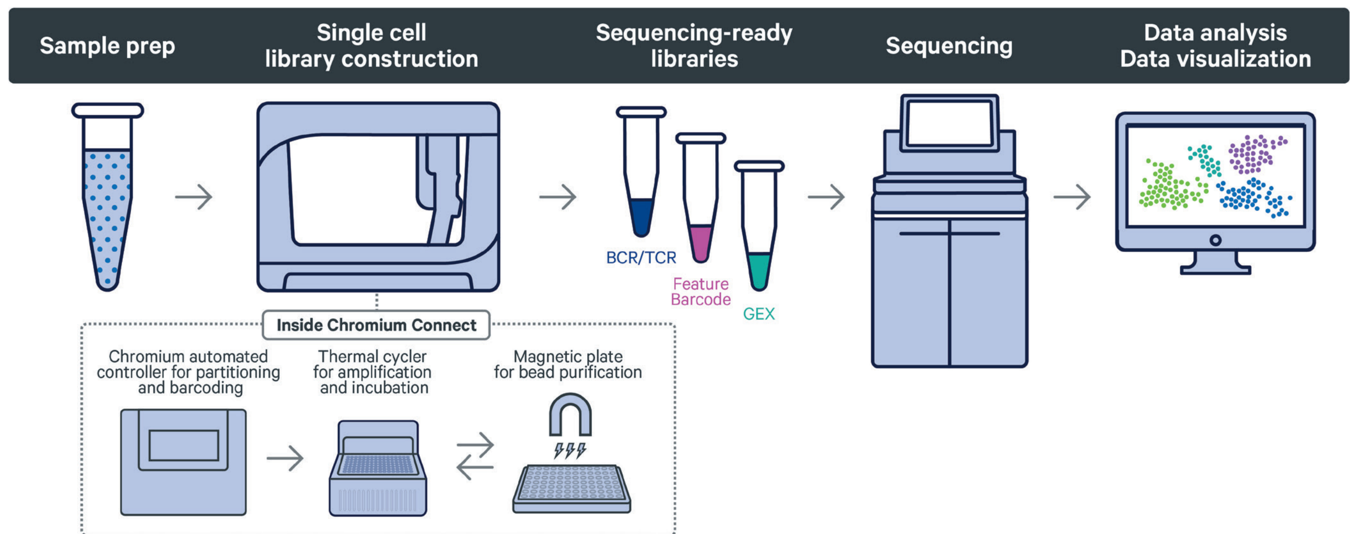

Figure 1. Overview of the Chromium Connect workflow for generating Chromium Next GEM Single Cell 5' v2 Gene Expression, V(D)J, and Cell Surface Protein libraries.

Data Highlight

Methods Overview

Chromium Next GEM Single Cell 5' v2 Gene Expression, Cell Surface Protein/Immune Receptor Mapping, and/or V(D)J (BCR or TCR) libraries were generated using the Chromium Connect automated workflow, and the manual workflow which incorporates the use of the Chromium Controller. Human Peripheral Blood Mononuclear Cells (PBMC) and human melanoma cells were labeled with BioLegend TotalSeqC TBNK antibody panel. Human Cytomegalovirus (CMV) T cells from a CMV+ donor were labeled with TotalSeqC TBNK antibody panel and two Immudex dCODE™ Dextramer® reagents. The targeted cell recovery for all cell types was 500-10,000 cells to enable side by side comparison. Melanoma cell samples were sorted by Fluorescence-Activated Cell Sorting (FACS) after antibody labeling. Cells from the same aliquot were used for both automated and manual workflows. At least two Chromium Connect instruments and one manual user were used in generating all libraries, including Cell Surface Protein libraries. Each instrument and manual user processed at least six replicates per run. The libraries were sequenced and the data were analyzed and visualized using Cell Ranger and Loupe Browser, respectively, as described in the respective user guides and the 10x Genomics Support website (see References).

Results

The results shown in Table 1 demonstrate that the Chromium Connect automated and manual workflows perform comparably in Single Cell 5' v2 Gene Expression library construction. The data shown in Figure 2 demonstrate that the automated and manual workflows capture similar Cell Surface Protein library sensitivity in different cell types. Cell type profiling for Single Cell 5' v2 Gene Expression and Cell Surface Protein Expression is consistent between both workflows (Figure 3). BCR and TCR transcripts derived from Single Cell 5' v2 V(D)J libraries show consistency in both the automated and manual workflows (Figures 4 and 5). A comparable fraction of antigen specific T cells with similar dextramer binding was identified in the data derived from Cell Surface Protein libraries generated using the automated and manual workflows (Figure 5).

Gene Expression Library Metrics	Human PBMCs (10,000 cells)		Human Melanoma cells (7,000 cells)		Anti-CMV T cells (5,000 cells)	
	Manual	Automated	Manual	Automated	Manual	Automated
Estimated number of cells	11469	9994	6652	4887	6584	5111
Fraction of reads in cells	92.8%	94.7%	96.9%	97%	77.5%	90.7%
Valid barcodes	89.2%	91.0%	91.4%	92.3%	94.4%	93.9%
Valid UMIs	99.5%	99.6%	99.7%	99.5%	99.6%	99.4%
Reads mapped confidently to transcriptome	52.8%	59.0%	64.5%	64.4%	73.9%	69.3%
Reads mapped confidently to genome	71.2%	79.1%	82.6%	84.2%	88.2%	88.1%
Reads mapped confidently to intronic regions	9.5%	11.3%	7.2%	9.3%	6.2%	9.4%
Reads mapped confidently to exonic regions	57.7%	64.0%	70.3%	69.9%	78.7%	75.1%
Median genes per cell (50,000 raw read pairs/cell)	1875	1921	1659	1727	2477	2941
Median UMI counts per cell (50,000 raw read pairs/cell)	5354	5304	5319	5319	6671	8474

Table 1. Comparable Chromium Next GEM Single Cell 5' v2 Gene Expression library metrics generated using either the automated or the manual Single Cell 5' v2 Gene Expression workflow. At least two Chromium Connect instruments and one manual user were used in the two workflows. At least six replicates were processed per run.

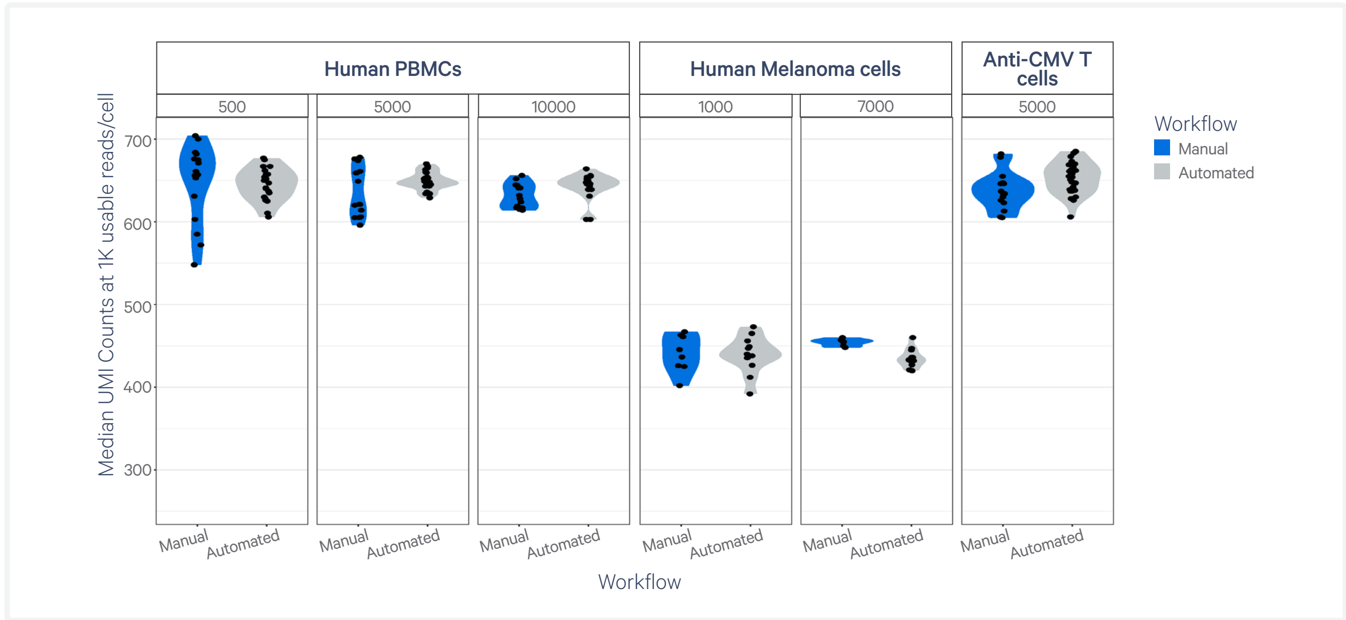

Figure 2. Chromium Next GEM Single Cell 5' v2 Cell Surface Protein libraries constructed using the automated and manual workflow show comparable library sensitivity as evident by the similar median UMI counts. Similar median UMI reads per cell were observed. Prior to library generation, all cells were labeled with BioLegend TotalSeqC TBNK antibody panel. In addition, anti-CMV T cells were labeled with two Immundex dCODE™ Dextramer® reagents. The Chromium Single Cell 5' v2 Cell Surface Protein datasets shown were down sampled to 1,000 usable read pairs per cell.

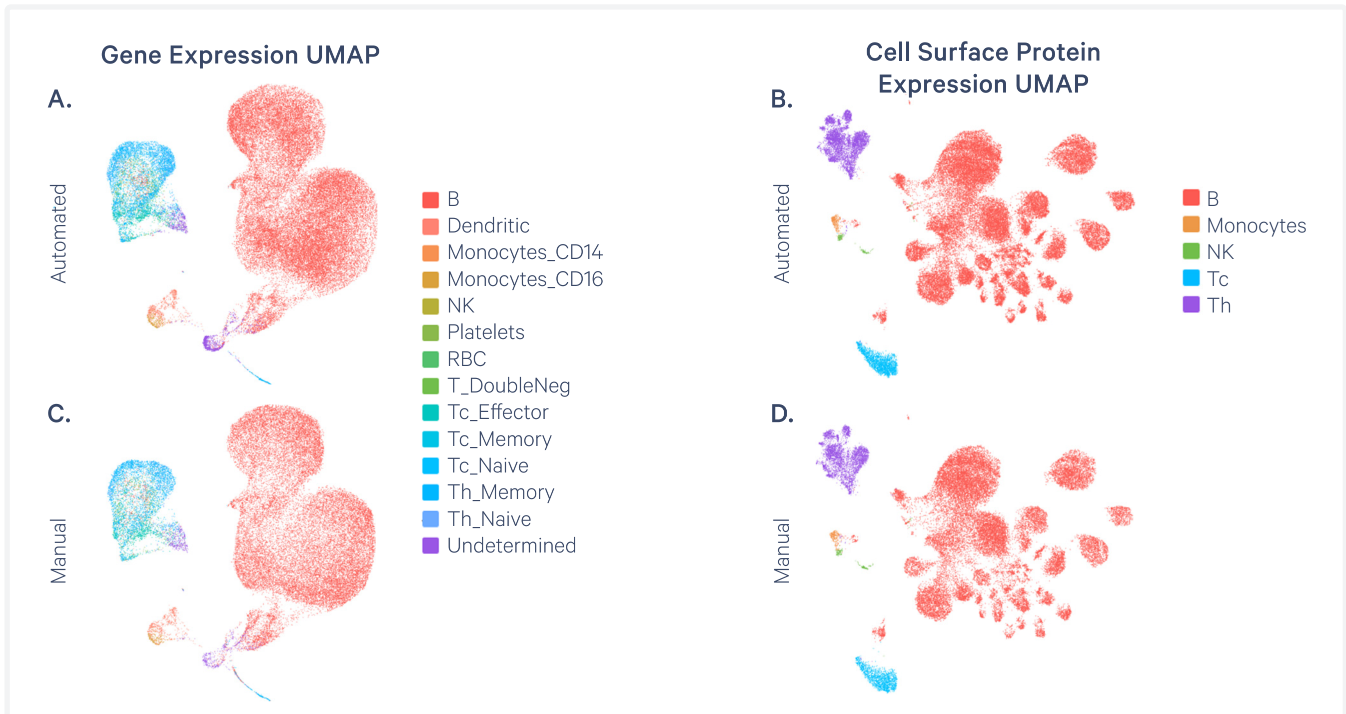

Figure 3. Consistent cell type profiling of human melanoma cells based on data derived from Chromium Next GEM Single Cell 5' v2 Gene Expression and Cell Surface Protein libraries generated using the automated and the manual workflows. (A) UMAP shows the gene expression data analyzed and visualized using Cell Ranger and Loupe Browser, respectively. Major immune cell populations in each cluster were identified via marker gene expression. 7,000 human melanoma cells and 12 libraries (constructed on three Chromium Connects) were analyzed. (B) Cell Surface Protein UMAP shows different cell types through cell surface protein expression after antibody labeling from the same cell population. (C) and (D) UMAPs show human melanoma cells Gene Expression and Cell Surface Protein libraries using the manual workflow. 7,000 human melanoma cells and six libraries (manually constructed by one user) were analyzed.

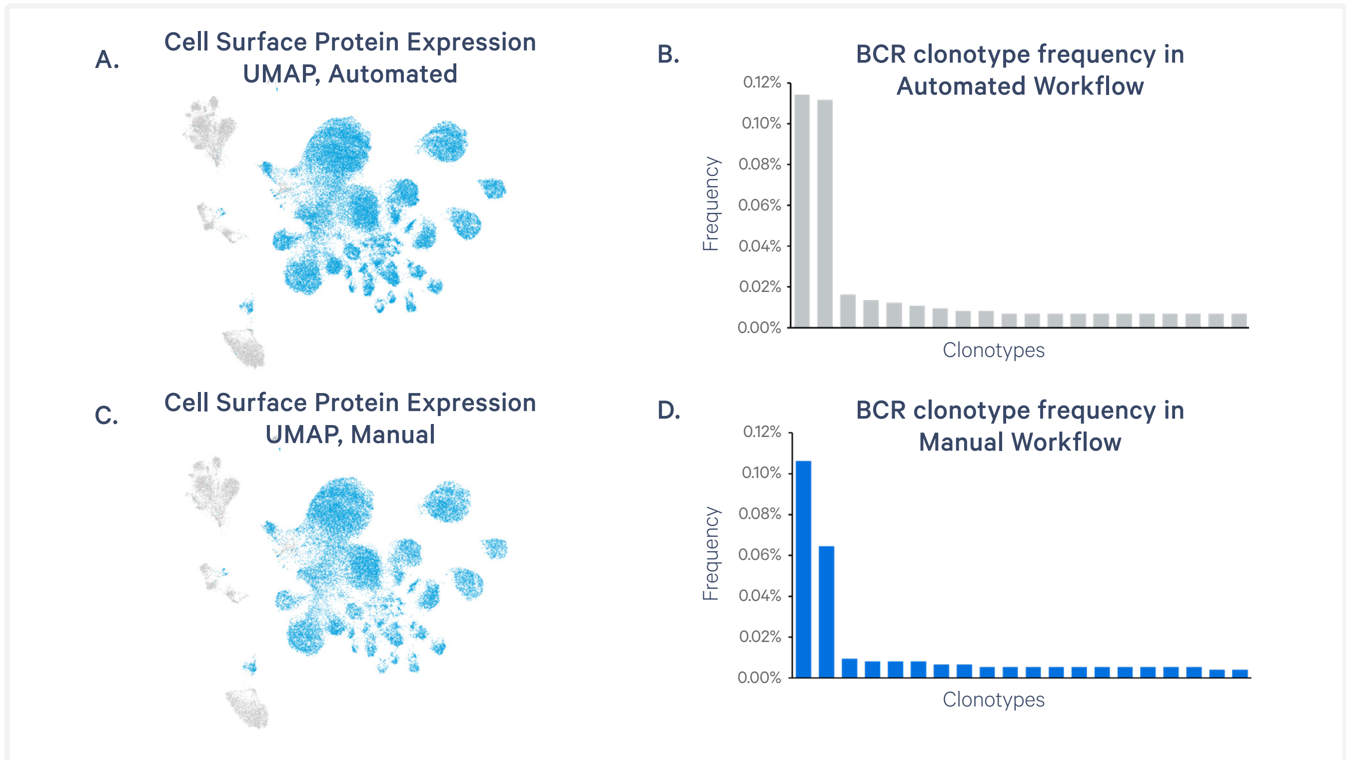

Figure 4. Consistent BCR transcript detection in Chromium Single Cell V(D)J libraries generated using the automated and manual Single Cell 5' v2 workflows. (A) Human melanoma cell surface protein UMAP derived from libraries generated using the automated workflow. Based on the BCR transcript detected in the Single Cell V(D)J libraries constructed from the pool of cells, cells highlighted in blue were assigned as B cells by Cell Ranger. This assignment is concordant with the B cells identified based on Cell Surface Protein expressoin (Figure 3B). 12 libraries were constructed on three Chromium Connect instruments, with a targeted cell recovery of 7,000 cells for each library. (B) A frequency distribution of the top 20 BCR clonotypes in data derived using the automated workflow. (C) and (D) Human melanoma cell surface protein UMAP and the top 20 BCR clonotype frequency in data generated using the manual workflow. Similarly, the cell calling based on BCR transcript and cell surface protein (Figure 3D) is highly consistent. Each library targets 7,000 human melanoma cells and six libraries (constructed by one manual user) are presented in the plot.

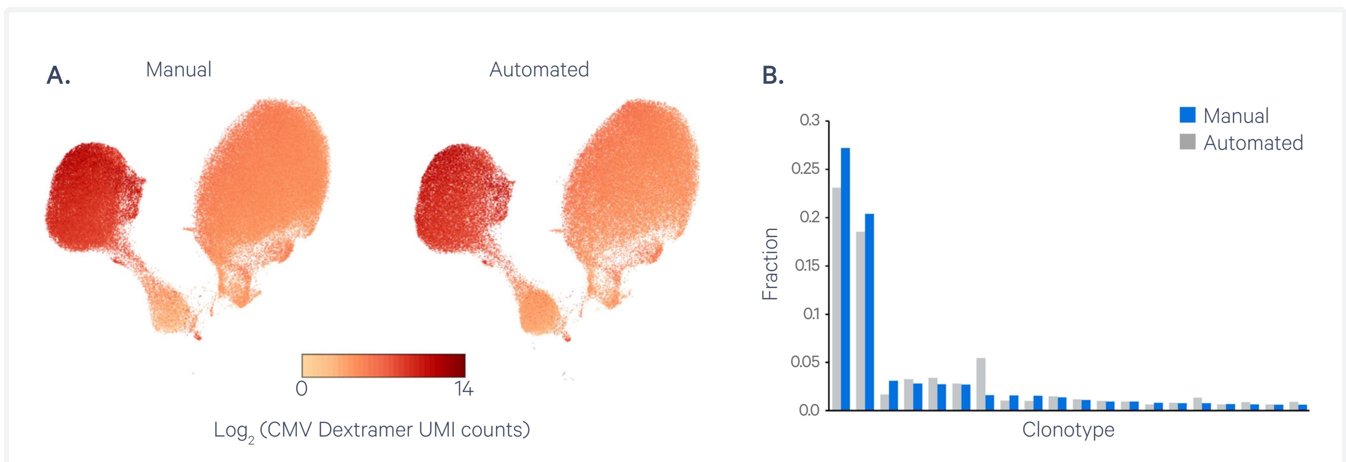

Figure 5. Multiomic characterization of 5000 anti-CMV T cells. Antigen specific TCR and expanded TCR clonotypes from automated and manual workflows detected from a CMV+ donor show comparable results. (A) UMAPs of Cell Surface Protein libraries show a similar fraction of antigen specific T cells in data derived from both workflows. CMV dextramer UMI counts are also comparable between the two workflows. Clusters were generated from 30 libraries (constructed on four Chromium Connect instruments) and 16 libraries (constructed by two manal users). (B) Top 20 TCR clonotype frequency distribution demonstrates consistent clonotype detection and relative abundance data for cell type profiling.

References

1. Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) User Guide (CG000331)
2. Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 User Guide (CG000384)
3. Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (CG000507)
4. Chromium Next GEM Automated Single Cell 5' cDNA Kit v2 Supplemental User Guide (CG000473)
5. Automated Gene Expression Library Construction User Guide (CG000474)
6. Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode technology (CG000149)
7. Cell Labeling with dCODE™ Dextramer® Reagents for Single Cell RNA Sequencing Protocols with Feature Barcode technology (CG000203)

Document Revision Summary

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