TECHNICAL NOTE

Chromium Connect: Consistent Automated Single Cell Gene Expression Library Generation

Introduction

Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input cell samples, minimizing technical variation in single cell gene expression data. Generation of Chromium Single Cell 3' Gene Expression libraries on the Chromium Connect instrument includes automated Gel Beads-in-emulsion (GEM) generation, barcoding, and library preparation from single cell suspensions, along with additional functionalities for library quantification and pooling. This Technical Note highlights the consistency and reproducibility of single cell gene expression data derived from libraries generated using the Chromium Connect automated workflow.

Chromium Connect Automated Workflow

The Chromium Connect automated workflow (Figure 1) generates Chromium Single Cell 3' Gene Expression libraries from up to 8 single cell samples per run, using Chromium Connect specific automation-compatible reagents and consumables with minimal user interaction. The libraries can be sequenced and the data analyzed and visualized using Cell Ranger and Loupe Cell Browser for assessing single cell gene expression.

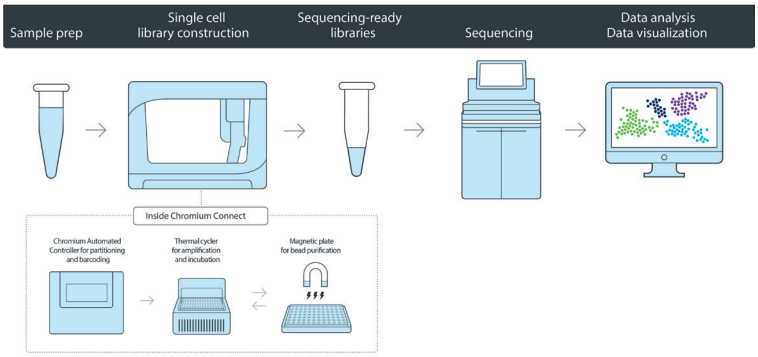


Figure 1. Chromium Connect with integrated components for an automated workflow for single cell library generation.

Methods

Peripheral Blood Mononuclear Cell (PBMC) single cell suspension was prepared as described in Demonstrated Protocol for Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing (Document CG00039). Cells were drawn from the same pool and Chromium Single Cell 3' Gene Expression libraries were generated targeting 10,000 cells/sample for each of the 8 channels, using either Chromium Connect instruments (up to 4) for the automated workflow or the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 protocol (Document CG000204) for the manual workflow.

Results

Highly consistent and reproducible data derived from Chromium Single Cell 3' Gene Expression libraries generated using the Chromium Connect automated workflow is shown in Table 1. A comparison of single cell gene expression data generated using the Chromium Connect automated workflow and the manual workflow is depicted in Table 2.

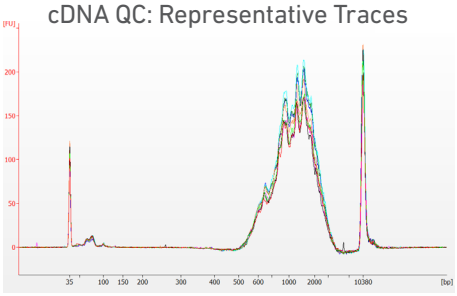
Table 1. Chromium Single Cell 3' Gene Expression libraries generated using the Chromium Connect automated workflow show highly consistent and reproducible data.

Automated Workflow–Data Consistency & Reproducibility

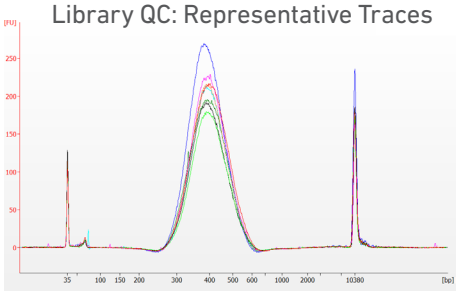
QC Traces: Channel-to-channel consistency

PBMC single cell suspension samples (1 replicate in each of the 8 channels in an instrument) were used to generate single cell gene expression libraries using the Chromium Connect automated workflow. Consistent cDNA QC and library QC traces were observed across all 8 channels of the chip used in the automated workflow, as shown in the representative Agilent BioAnalyzer traces. All sample traces showed comparable size distribution and concentration, indicating highly consistent performance.

cDNA QC: Representative Traces

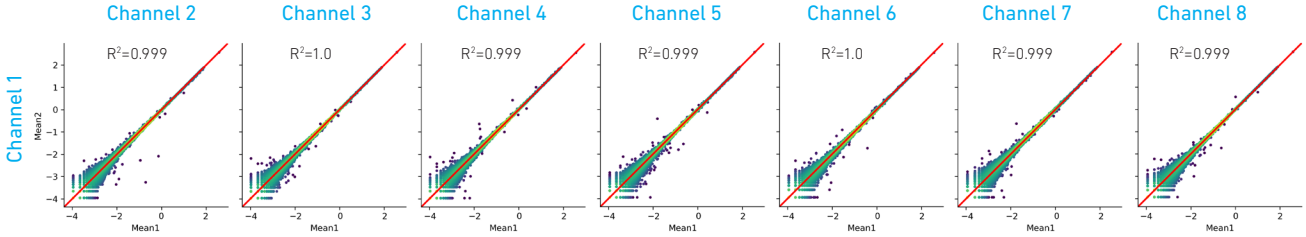


Library QC: Representative Traces



Gene Expression Profile: Channel-to-channel consistency

Each of the libraries generated across the 8 channels of the Chromium Connect instrument showed highly consistent gene expression profiles. As shown below, the correlation value of gene expression profile of libraries derived form each channel relative to channel 1 was >0.99.



Gene Expression Profile: Instrument-to-instrument consistency

The gene expression profiles of PBMCs used for generating single cell gene expression libraries across 4 different Chromium Connect instruments showed high degree of consistency and correlation.

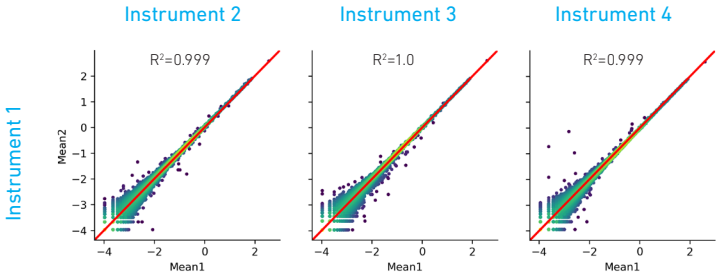
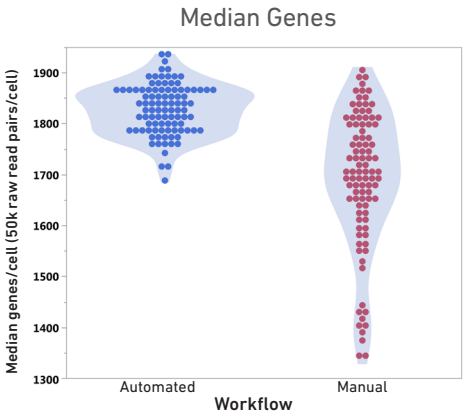


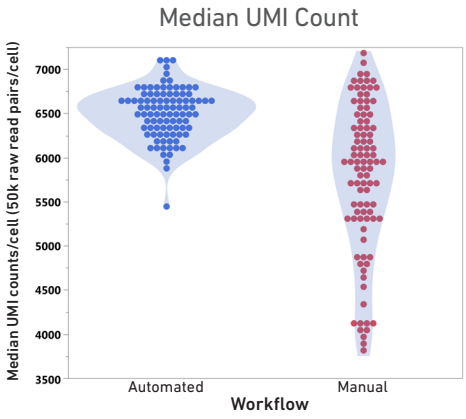
Table 2. Comparison of data derived from Chromium Single Cell 3' Gene Expression libraries generated using either the Chromium Connect automated workflow or the manual workflow.

Single Cell Gene Expression Data—Automated vs. Manual Workflow

Minimal User Variability

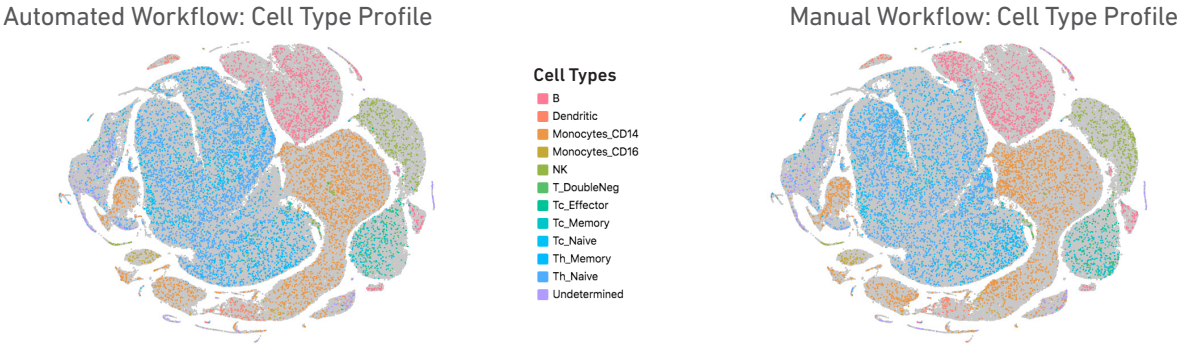
The Chromium Connect automated hands-off workflow minimizes user variability. Differences in user handling likely contribute to a wider distribution of median genes and Unique Molecular Identifier (UMI) counts recovered per cell in 96 libraries prepared using the manual workflow compared to 96 libraries generated using the Chromium Connect automated workflow (over multiple days, targeting 500-10,000 cells/sample).





Consistent Cell Type Profiling

The gene expression profiles compared in libraries generated from 10,000 PBMCs using either the manual or the Chromium Connect automated workflow identifies all major PBMC cell types. The single cell barcode tSNE plots show the gene expression data analyzed and visualized using Cell Ranger and Loupe Cell Browser, respectively. Major cell populations in each cluster were identified via marker gene expression. Comparable cell type profile among the two sets of data is also shown in the graph below. This demonstrates that the automated workflow generates consistent gene expression data for cell type profiling.



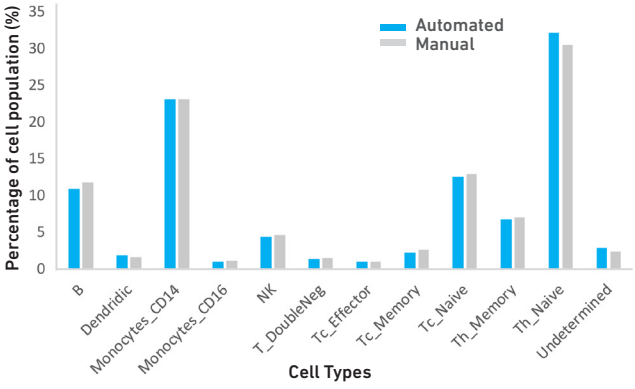
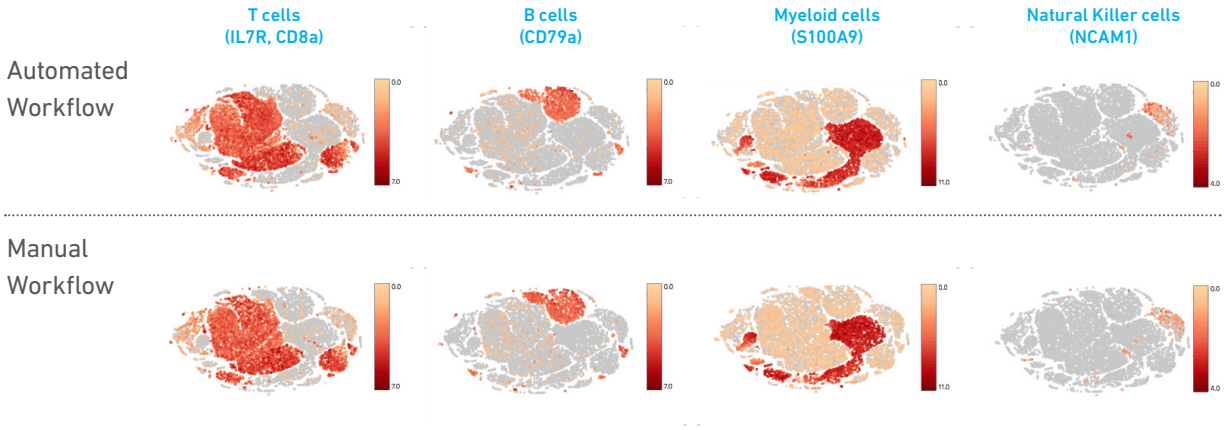


Table 2 contd. Comparison of data derived from Chromium Single Cell 3' Gene Expression libraries generated using either the Chromium Connect automated workflow or the manual workflow.

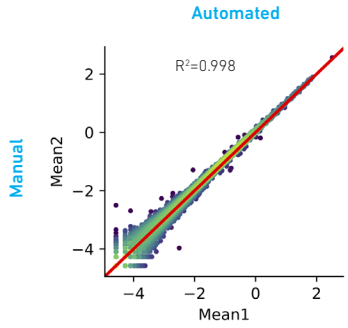
Single Cell Gene Expression Data—Automated vs. Manual Workflow

Accurate Cell Type Marker Detection

To investigate possible changes in gene expression due to automated workflow, expression of major blood cell type markers were compared in data generated using either the automated or the manual workflows. All the key markers were detected in both datasets (see t-SNE plots below).



Comparison of cell type marker expression data generated from 10,000 PBMCs using 4 channels in one Chromium Connect run highly correlated with the data generated using the manual workflow (4 channels/run by 1user) with a correlation value of >0.99, as shown in the plot below.



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