TECHNICAL NOTE

Chromium Connect: Consistent Automated Single Cell 5' Gene Expression and V(D)J Library Generation

Introduction

Chromium Connect automates the preparation of sequencing-ready, single cell 5' Gene Expression and V(D)J libraries from the same input cell sample, minimizing technical variation in single cell gene expression data and immune repertoire profiling. Generation of Chromium Single Cell 5' Gene Expression and V(D)J 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 and V(D)J 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 5' Gene Expression and V(D)J libraries for 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 Browser for assessing the immune repertoire and single cell gene expression.

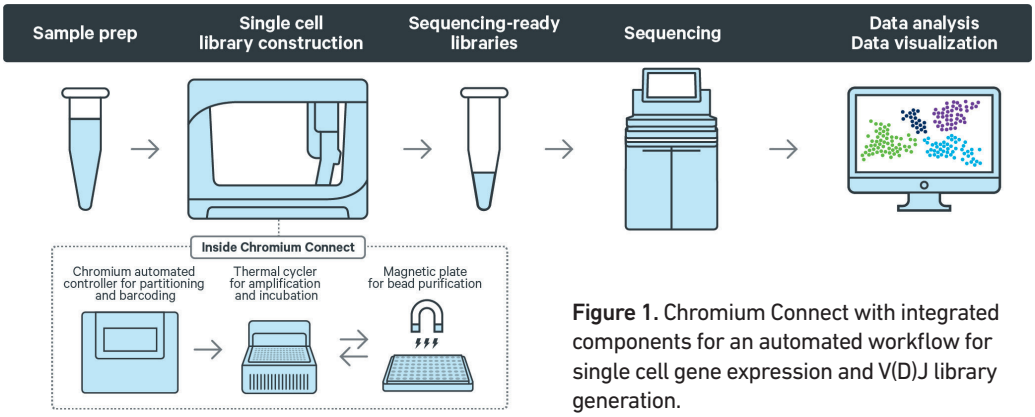

Figure 1. Chromium Connect with integrated components for an automated workflow for single cell gene expression and V(D)J library generation.

Methods

Human 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 5' Gene Expression and V(D)J libraries were generated targeting 5,000 cells/sample for 4-6 channels, using either Chromium Connect instruments (up to 2) for the automated workflow or the Chromium Next GEM Single Cell 5' Reagent Kits v2 protocol (Document CG000331) for the manual workflow.

For the V(D)J clonotype detection, cDNA derived by targeting 5,000 PBMCs/sample was used to generate V(D)J libraries using either Chromium Connect instruments (up to 2) for the automated workflow or the Chromium Next GEM Single Cell 5' Reagent Kits v2 protocol (Document CG000331) for the manual workflow.

Results

Highly consistent and reproducible data derived from Chromium Single Cell 5' Gene Expression libraries generated using the Chromium Connect automated workflow is shown in Figures 2-3. A comparison of single cell gene expression data generated using the Chromium Connect automated workflow and the manual workflow is depicted in Figures 4-6. Comparison of integrated gene expression and V(D)J data generated using the automated and manual workflow is shown in Figure 7. V(D)J data consistency and reproducibility using the automated workflow is shown in Figure 8, while Figure 9 shows comparable V(D)J clonotype detection in manual and automated workflows.

Automated Workflow–Data Consistency & Reproducibility

PBMC single cell suspension samples (same sample across 4-6 channels on an instrument) were used to generate single cell gene expression libraries using the Chromium Connect automated workflow. Chromium Single Cell 5' Gene Expression libraries generated using the Chromium Connect automated workflow show highly consistent and reproducible data (Figures 2 and 3).

QC Traces: Channel-to-channel consistency

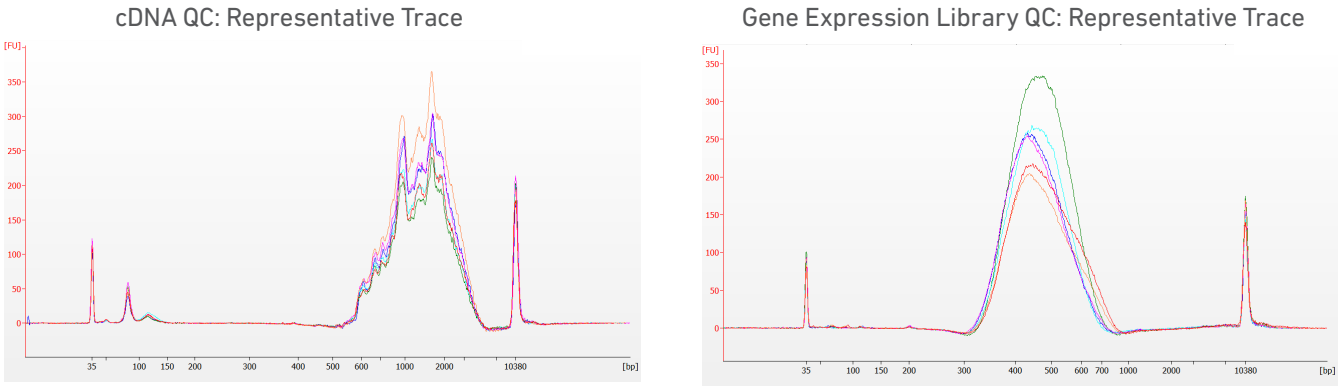

Figure 2. Consistent cDNA and gene expression library QC traces were observed across all channels of the chip used, as shown in the representative Agilent BioAnalyzer traces. All sample traces showed comparable size distribution and concentration, indicating highly consistent performance.

Gene Expression Profile: Instrument-to-instrument consistency

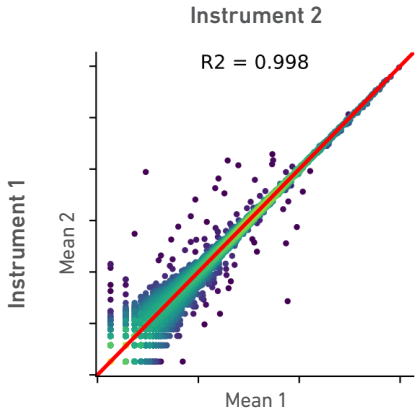

Figure 3. The single cell gene expression profiles obtained from libraries prepared across 2 Chromium Connect instruments show high degree of consistency and correlation.

Single Cell Gene Expression Data—Automated vs. Manual Workflow

A comparison of data derived from Chromium Single Cell 5' Gene Expression libraries generated using either the Chromium Connect automated workflow or the manual workflow is shown in Figures 4-6.

Minimal User Variability

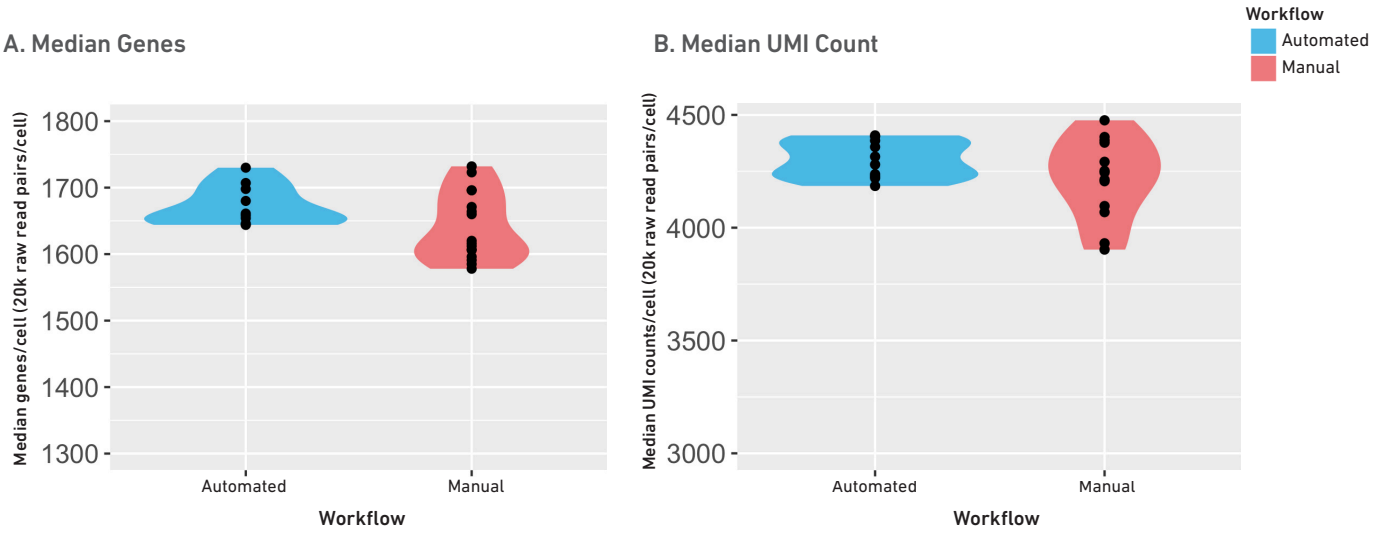

Figure 4. The Chromium Connect automated, hands-off workflow minimizes user variability. Differences in user handling likely contribute to a wider distribution of median genes **(A)** and UMI counts recovered per cell **(B)** in 16 libraries prepared using the manual workflow compared to 10 libraries generated using the Chromium Connect automated workflow.

Consistent Cell Profiling

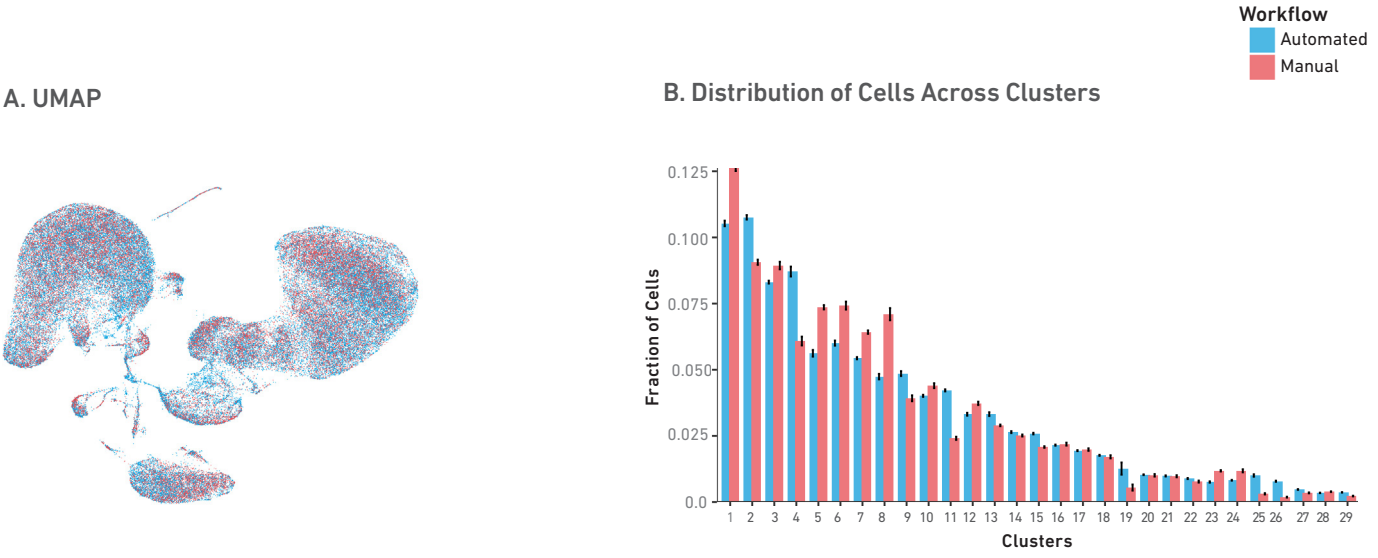

Figure 5. Single cells were clustered using a graph-based clustering algorithm. Libraries generated using either Chromium Connect automated or manual workflow have comparable transcriptomic profiles as evident by their overlap in the aggregated UMAP **(A)** and comparable distribution for each cell cluster **(B)**.

Consistent Cell Type Profiling

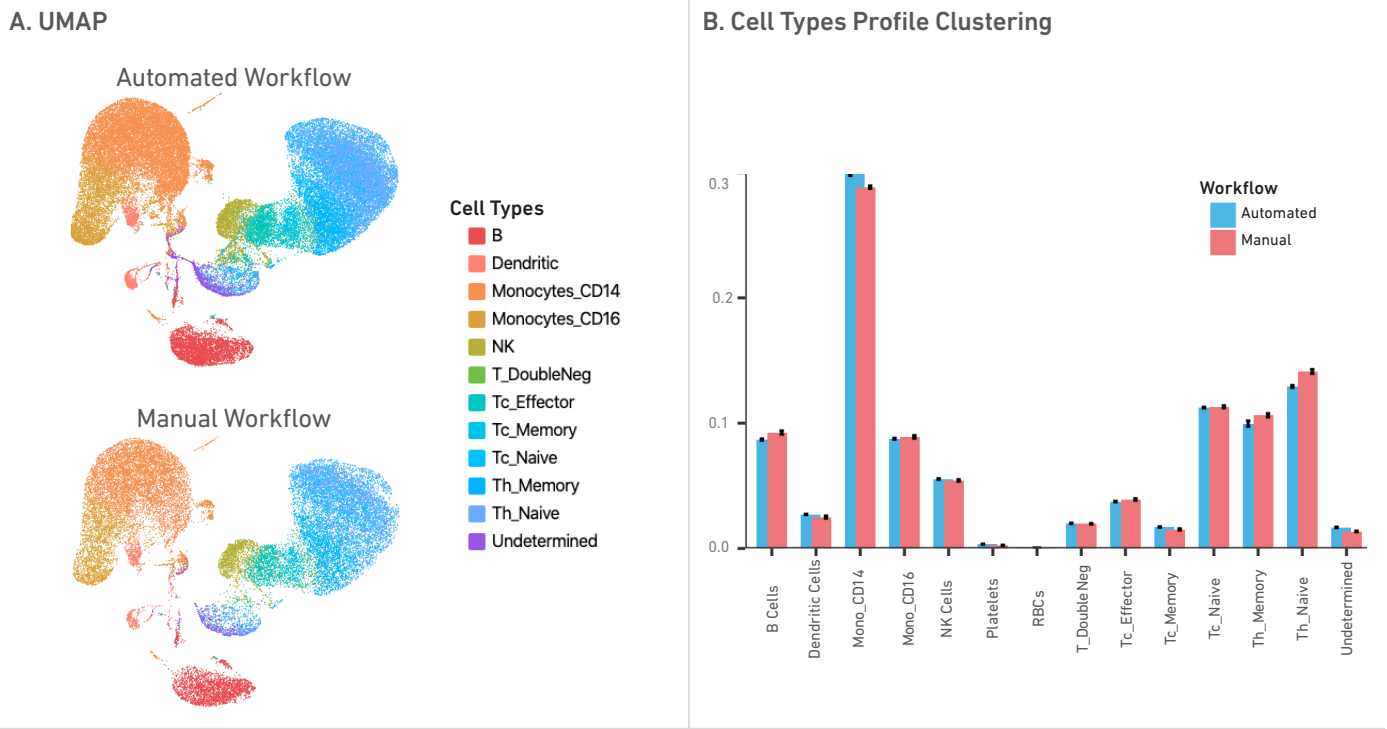

Figure 6. The gene expression profiles from 6 libraries generated using the manual workflow and 10 libraries generated using the Chromium Connect automated workflow identifies all major cell types in human PBMCs. A. The UMAP shows the gene expression data analyzed and visualized using Cell Ranger and Loupe Browser, respectively. Major cell populations in each cluster were identified via marker gene expression. **B.** Comparable cell type profile among the two sets of data is shown in the graph above, demonstrating consistent gene expression data for cell type profiling.

Single Cell Gene Expression, TCR and BCR Data—Automated vs. Manual Workflow

Data from gene expression, TCR, and BCR libraries were integrated for both Chromium Connect automated and manual workflows.

Multidimensional Immune Profiling

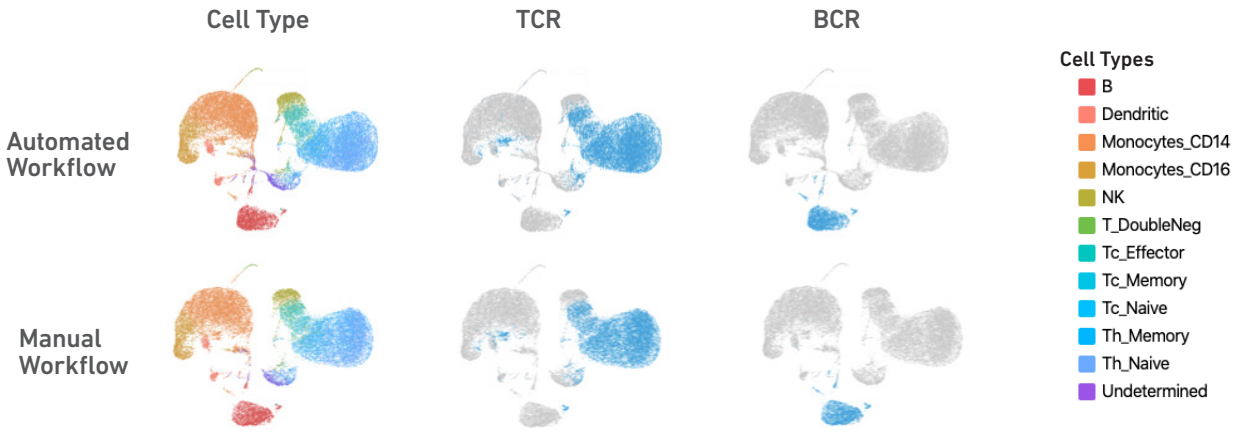

Figure 7. TCR and BCR clonotypes overlap with T cell and B cell compartment, respectively. UMAPs are colored by inferred cell types, TCR clonotypes, and BCR clonotypes.

Automated Workflow - V(D)J Data Consistency & Reproducibility

A comparison of data derived from Chromium Single Cell V(D)J libraries generated using either the Chromium Connect automated workflow or the manual workflow shows comparable TCR/BCR library complexity.

V(D)J Sequences Recovered & library Complexity

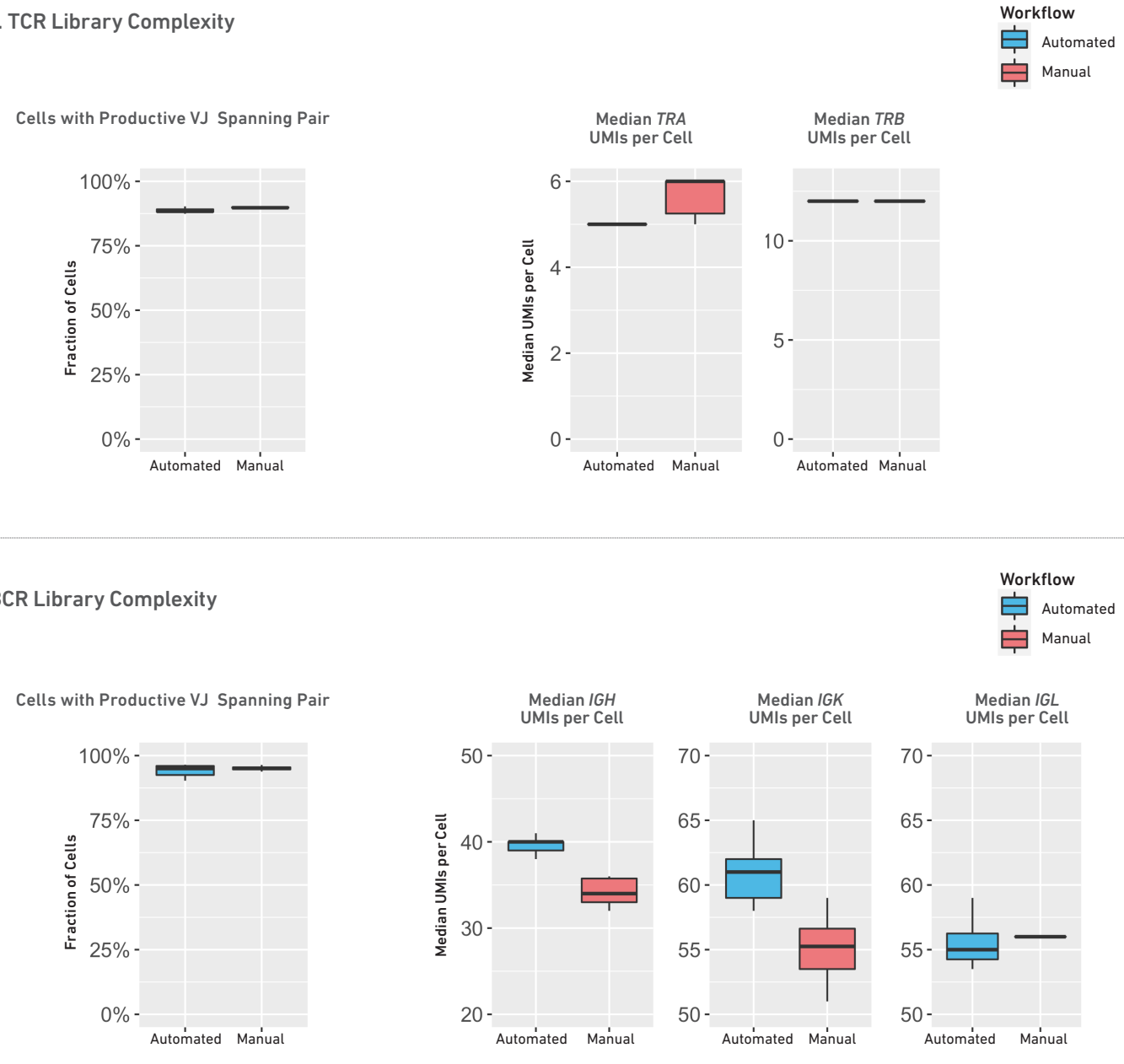

Figure 8. Similar fraction of cells with productive VJ spanning pair were detected in both workflows. The Chromium Connect automated workflow yielded similar sensitivity for both *TRA* and *TRB* **(A)**, as well as for *IGH*, *IGK*, and *IGL* **(B)** when compared to the data from the manual workflow.

V(D)J Amplification Data—Automated vs. Manual Workflow

To determine if similar V(D)J clonotypes could be detected with the automated workflow, cDNA derived from 5,000 PBMCs captured using the manual workflow was used to generate V(D)J libraries using either the manual or the Chromium Connect automated workflow.

Consistent Clonotype Detection

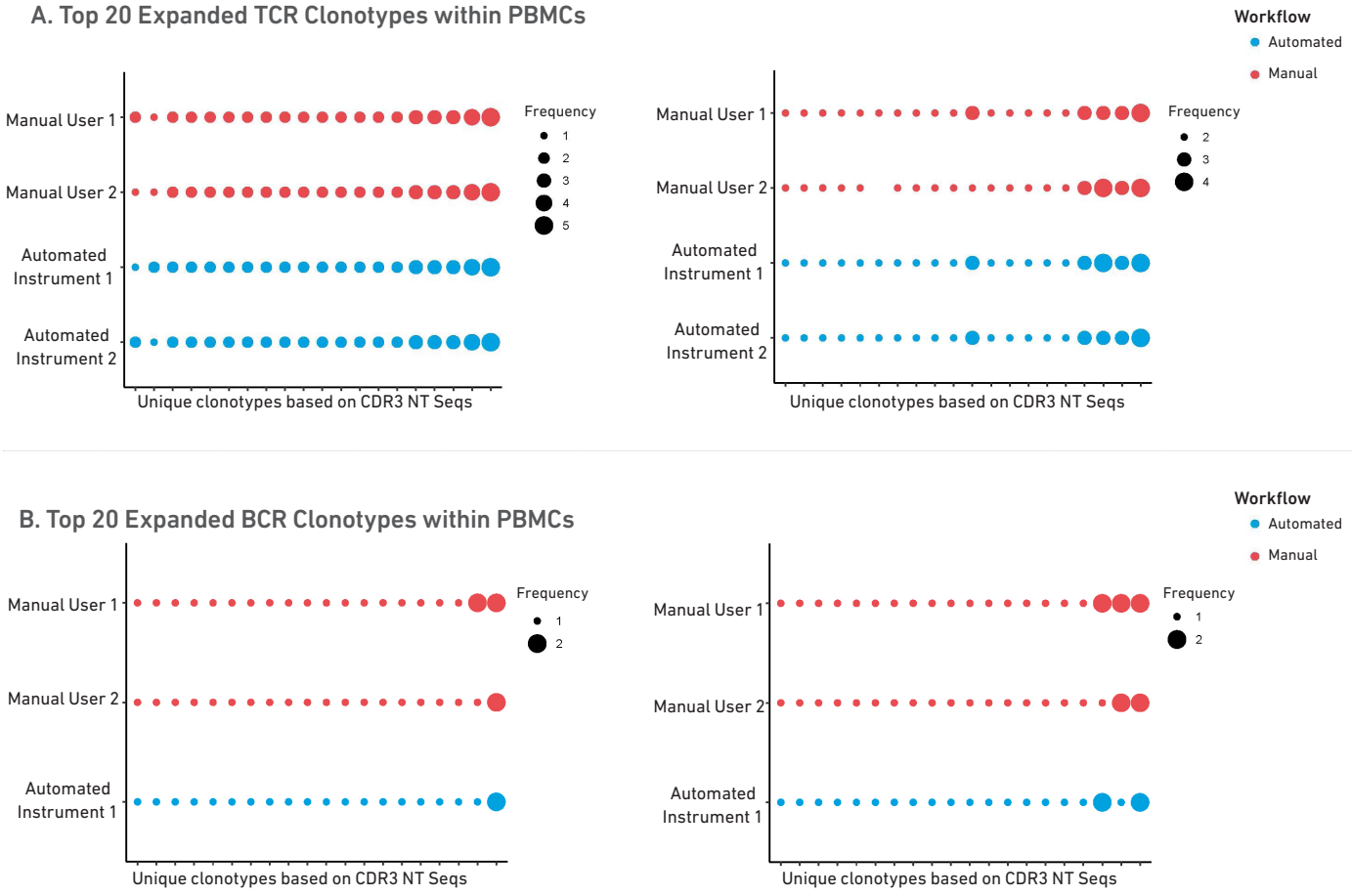

Figure 9. V(D)J Clonotype data derived from Chromium Single Cell V(D)J libraries generated using either the Chromium Connect automated or the manual workflow. Representative data from two cDNA samples are shown above. The top 20 most expanded clonotypes identified in libraries generated from the same cDNA show that Chromium Connect automated workflow offers consistent clonotype detection.

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