Chromium Connect: Automated Single Cell cDNA Yield & Library Metrics Overview

Introduction

Chromium Connect automates the preparation of sequencing-ready, single cell libraries from input cDNA generated from samples. The cDNA and corresponding library total mass yields for automated Chromium Single Cell 3' Gene Expression, 5' Gene Expression, and V(D)J workflows on Chromium Connect are ~50-80% lower compared to respective manual workflows. This Technical Note highlights that despite the lower yields, the key performance metrics of these libraries are comparable to the libraries constructed manually.

Methods Overview

Chromium Next GEM Single Cell 3' v3.1 Gene Expression, 5' v2 Gene Expression, and V(D)J (BCR or TCR enriched) libraries were generated using the Chromium Connect automated workflows and the corresponding manual workflows. Human Peripheral Blood Mononuclear Cells (PBMC), Cytomegalovirus (CMV) infected T cells, and Melanoma Dissociated Tumor Cells (DTCs) were used for generating the various libraries.

The cDNA and gene expression library concentrations were measured using Agilent BioAnalyzer as per manufacturer's instructions. The libraries were sequenced and the data were analyzed using Cell Ranger as described in the respective user guides (see References) and the 10x Genomics Support website.

Results

In each of the representative datasets generated from the automated workflow and the corresponding manual workflow, the automated workflow produced cDNAs and libraries with lower concentrations compared to the manual workflow.

In the Single Cell 3' v3.1 Gene Expression dataset from 10,000 human PBMCs, automated cDNA and gene expression library concentrations are 50.8% and 62.9% of corresponding products generated manually (Figure 1A-1B).

In the Single Cell 5' v2 Gene Expression and V(D)J (TCR enriched) library dataset from 5,000 CMV infected T cells, automated cDNA and gene expression library concentrations are 58.0% and 51.3% of corresponding products generated manually (Figure 2A-2B).

In the Single Cell 5' v2 Gene Expression and V(D)J (BCR enriched) library dataset from 1,000 Melanoma DTCs, automated cDNA and gene expression library concentrations are 13.5% and 39.1% of corresponding products generated manually (Figure 3A-3B).

Despite lower cDNA and library yields from the automated workflows, the key library metrics between manual and automated workflows are comparable. Specifically, at the whole sample level, a comparable number of recovered cells are observed for both Single Cell 3' v3.1 and 5' v2 assays (Figure 1C, 2C, 3C). At the individual cell level, comparable library complexity was indicated by similar median genes and median UMI counts per cell (Figure 1D, 2D, 3D). In addition, the numbers of T or B cells detected in the V(D)J libraries are also comparable between the automated and manual workflows (Figure 2F, 3F).

Further, Single Cell Gene Expression and V(D)J libraries can be consistently generated from cDNAs as low as 100 - 150 pg/µl. An example of cDNA and library traces (Figure 4) show that the low concentration of cDNA generated using the automated workflow does not impact Single Cell 5' v2 Gene Expression and V(D)J TCR enriched library construction.

Conclusions

In summary, the data presented in this Technical Note demonstrate that despite the lower cDNA and library yields in the automated workflow, the Single Cell Gene Expression and V(D)J library metrics are comparable to the libraries generated using the manual workflow. Hence, cDNA and library yields are not the primary predictors of library quality and complexity.

Figure 1. Single Cell 3' v3.1 Gene Expression assay: comparison of cDNA yield, library yield, and library metrics for automated and manual workflows.

Generated from 10,000 human PBMCs, cDNA and libraries from automated workflow (n=16, generated on 2 Chromium Connects) have significantly lower concentration compared to those generated using the manual workflow (n=16, generated by 2 manual users) (A-B).

Libraries generated using the automated and manual workflows show comparable cell recovery, median genes, and median UMI counts per cell (C-E), indicative of similar library complexity and sensitivity. All libraries shown are down sampled to 50,000 raw read pairs per cell.

Figure 2. Single Cell 5' v2 Gene Expression and V(D)J (TCR enriched) assay: comparison of cDNA yield, library yield, and library metrics for automated and manual workflows.

Generated from 5,000 CMV infected T cells, cDNA and libraries from automated workflow (n=16, generated on 2 Chromium Connects) have significantly lower concentration compared to those generated using the manual workflow (n=8, generated by 1 manual user) (A-B).

Libraries generated using the automated and manual workflows show comparable cell recovery, median genes, and median UMI counts per cell (C-E), indicative of similar library complexity and sensitivity. Data derived from Single Cell V(D)J TCR enriched libraries showed comparable T cell recovery and cell calling between automated and manual workflows (F). All libraries shown were down sampled to 50,000 raw read pairs per cell.

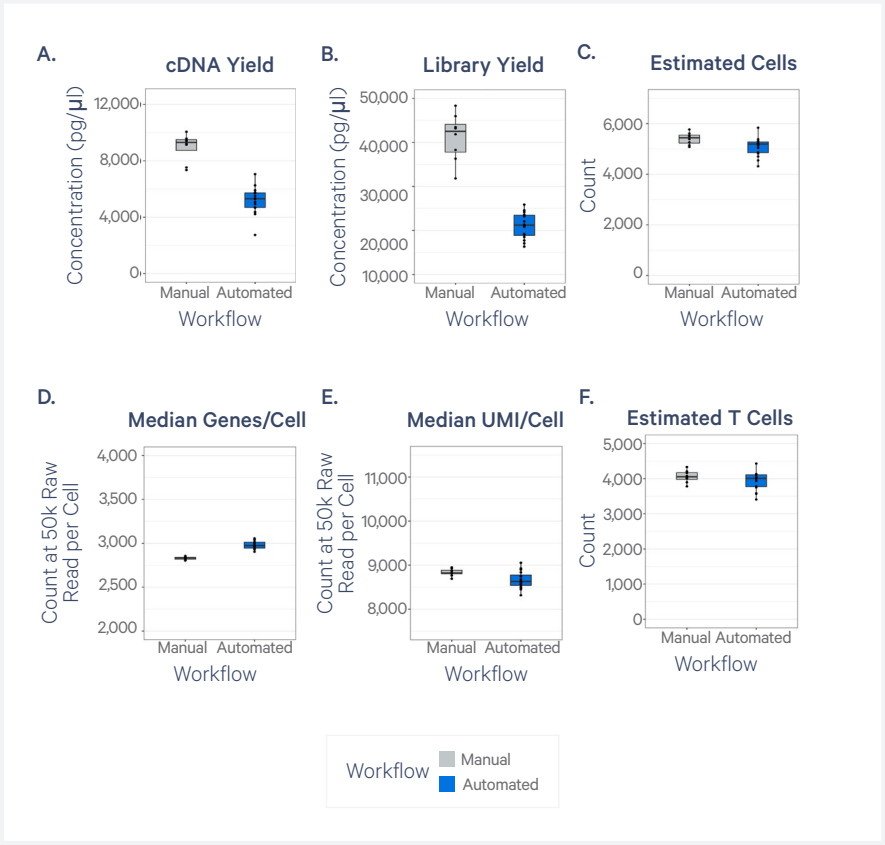
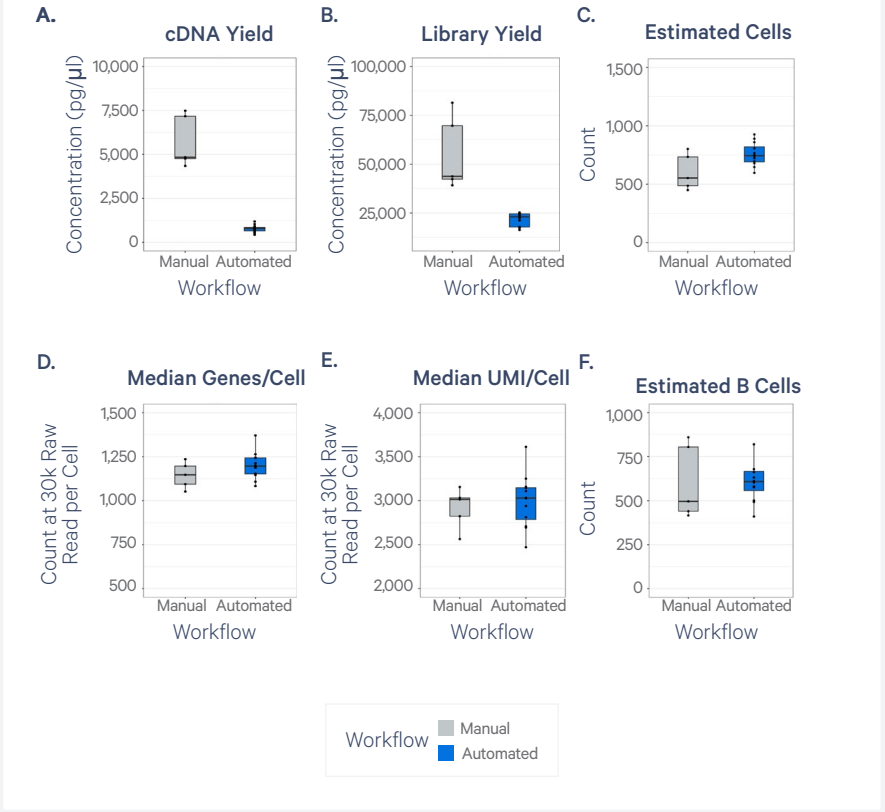


Figure 3. Single Cell 5' v2 Gene Expression and V(D)J (BCR enriched) assay: comparison of cDNA yield, library yield, and library metrics for automated and manual workflows.

Generated from 1,000 Melanoma DTCs, cDNA and libraries from automated workflow (n=16, generated on 2 Chromium Connects) have significantly lower concentration compared to those generated using the manual workflow (n=5, generated by 1 manual user) (A-B).

Libraries generated using the automated and manual workflows show comparable cell recovery, median genes, and median UMI counts per cell (C-E), indicative of similar library complexity and sensitivity. All libraries shown are down sampled to 30,000 raw read pairs per cell.

Data derived from Single Cell V(D)J BCR enriched libraries showed comparable B cell recovery and cell calling between automated and manual workflows (F). Libraries shown were down sampled to 50,000 raw read pairs per cell.



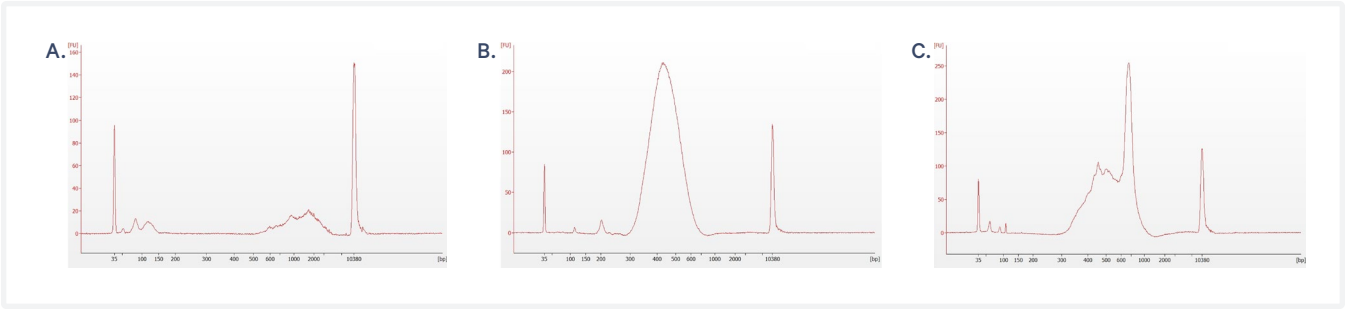


Figure 4. Representative cDNA and library traces. cDNA was generated from 500 human PBMCs on Chromium Connect as per the Single Cell 5' v2 Gene Expression and V(D)J workflow. Samples were run on an Agilent Bioanalyzer High Sensitivity chip. The cDNA trace (A) was used to calculate concentration of 121.94 pg/µl. The cDNA was used to generate Single Cell 5' v2 Gene Expression and V(D)J TCR enriched libraries of expected size (B-C). The library yields were sufficient for sequencing and the library performance metrics were comparable to libraries generated from higher concentration cDNA (data not shown).

References

1. Chromium Connect Instrument User Guide (CG000180)
2. Chromium Next GEM Automated Single Cell 3' v3.1 User Guide (CG000286)
3. Chromium Next GEM Automated Single Cell 5' User Guide (CG000384)

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