Chromium Connect: Single Cell 3' Gene Expression Dual Index Library Data Overview

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. This Technical Note highlights the consistency and reproducibility in generation of dual index Chromium Single Cell 3' Gene Expression libraries using the Chromium Connect automated workflow. The documents also presents a comparison of representative data derived from dual versus single index Chromium Single Cell 3' libraries generated using the automated workflow.

Automated Chromium Single Cell 3' Dual Index Library Generation

Chromium Single Cell 3' Gene Expression libraries with dual indices can now be also generated using the Chromium Connect automated workflow and reagents. These libraries are similar to Automated Single Cell 3' Gene Expression single index libraries , but in addition to the standard P5, P7, i7 sample index, Read 1, and Read 2 sequences that flank the 10x Barcode, UMI, and insert, the dual index libraries also include an i5 sample index (Figure 1).

These sequencing-ready dual index libraries can be pooled and sequenced based on recommendations outlined in Chromium Next GEM Single Cell 3' user guide (Document CG000286).

The data can be analyzed and visualized using Cell Ranger and Loupe Cell Browser for assessing single cell gene expression. Cell Ranger analysis pipeline "cellranger mkfastq", supports demultiplexing of the dual index libraries, ensuring that reads without a specified pair of dual indices are not assigned to a sample.

Index hopping is a phenomenon that occurs during cluster generation of libraries on the sequencing flow cell and can result in incorrect assignment of insert (RNA) reads to a sample. Dual indexing mitigates index hopping during demultiplexing by enabling the computational identification of reads that contain an expected pair of unique i5 and i7index sequences. Typically 0.1-2% of reads are filtered out due to index hopping, where an invalid pair of index sequences is assigned to a given sample read. See Technical Note (Document CG000325).

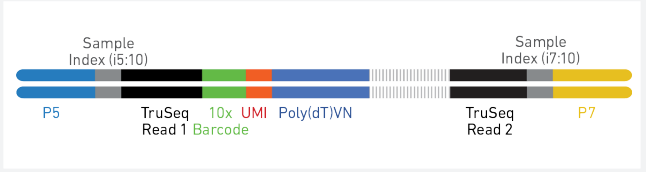

Figure 1. A representative Chromium Single Cell 3' Gene Expression dual index library schematic with sample index i5 and i7 generated using an automated workflow on Chromium Connect.

Chromium Connect automated workflow can now generate Chromium Single Cell 3' Gene Expression dual index libraries from up to 8 single cell samples per run, using Chromium Connect specific automation-compatible reagents and consumables with minimal user interaction. The automated workflow for generating dual index libraries is similar to the workflow for single index libraries, except that a dual index plate is used instead of a single index plate for sample indexing.

A "DUAL INDEX" icon is placed adjacent to the dual indexing reagents and steps in the relevant user guide. An illustrative overview of dual sample index addition steps with corresponding sample index plate along with the final library schematic is shown below (Figure 2). The manual workflow for generating Chromium Single Cell 3' Gene Expression dual index libraries also uses the same dual index plate for sample index PCR (Document CG000325).

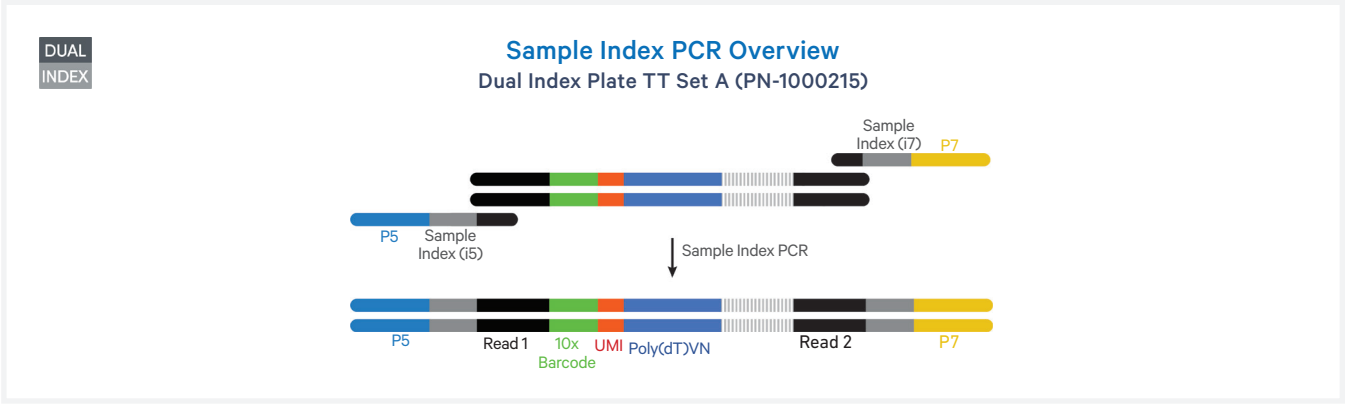

Figure 2. Sample index overview showing the dual index plate used for generating Chromium Single Cell 3' Gene Expression dual index library on Chromium Connect.

Method Overview

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. Either Chromium Single Cell 3' Gene Expression dual index or single index libraries were generated targeting 500, 1,000 or 10,000 cells/ sample for each of the 8 channels on Chromium Connect instrument (up to 4 instruments for dual index and 4 instrument for single index libraries). The libraries were sequenced as shown in Table 1. The sequencing data were analyzed and visualized using Cell Ranger 6.1 and Loupe 4.5.1.

Chromium Single Cell 3' Gene Expression	
Dual Index	**Single Index**
Sequencing Depth	
Minimum 20,000 read pairs per cell	Minimum 20,000 read pairs per cell
Sequencing Type	
Paired-end, dual indexing	Paired-end, single indexing
Sequencing Read	
Read 1: 28 cycles i7 Index: 10 cycles i5 Index: 10 cycles Read 2: 90 cycles	Read 1: 28 cycles i7 Index: 8 cycles i5 Index: 0 cycles Read 2: 91 cycles

Table 1. Sequencing recommendations for Chromium Single Cell 3' Gene Expression dual index and single index libraries.

Representative Data

The representative data in figures 3-6 show comparison of key results derived from Peripheral Blood Mononuclear Cells (PBMCs). The results clearly demonstrate that both single and dual index versions of the Chromium Single Cell 3' Gene Expression libraries generated using Chromium Connect automated workflow yield comparable cell clustering, library correlation, mapping rates, and gene expression. The data were consistent across three different targeted cell recovery of 500, 1,000, and 10,000 cells per sample.

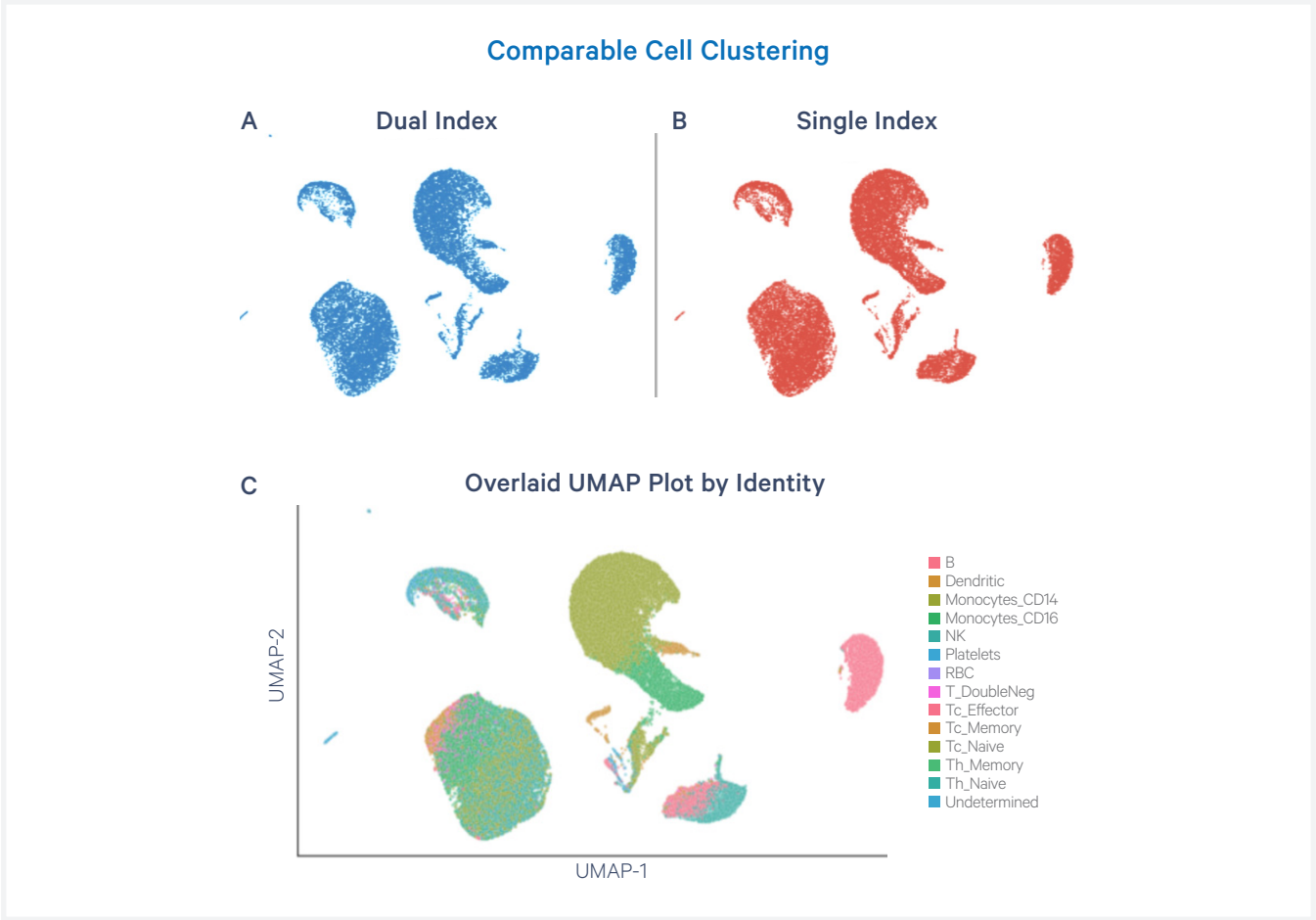

Figure 3. Chromium Single Cell 3' Gene Expression dual and single index libraries generated using the automated Chromium Connect workflow show comparable clustering and overlapping cell populations. A. UMAP plot generated from 1,000 PBMCs profiled using the Single Cell 3' Gene Expression v3.1 automated workflow (Dual Index). B. UMAP plot generated from 1,000 PBMCs profiled using the Single Cell 3' Gene Expression v3.1 automated workflow (Single Index). C. Aggregated UMAP plot of both chemistries indicates comparable cell clustering and cell subpopulation detection.

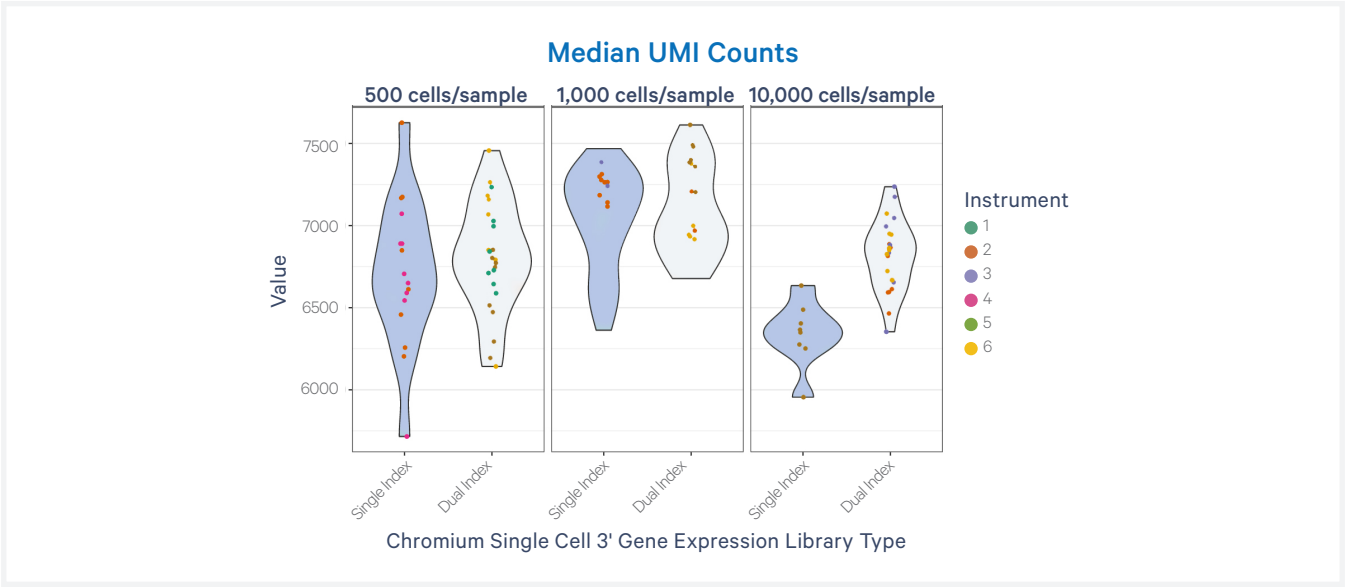

Figure 4. Data derived from Chromium Single Cell 3' Gene Expression dual and single index libraries generated using the Chromium Connect automated workflow (across multiple instruments) show comparable Unique Molecular Identifier (UMI) counts recovered per cell in data derived from PBMCs (targeting 500, 1,000 and 10,000 cells/sample).

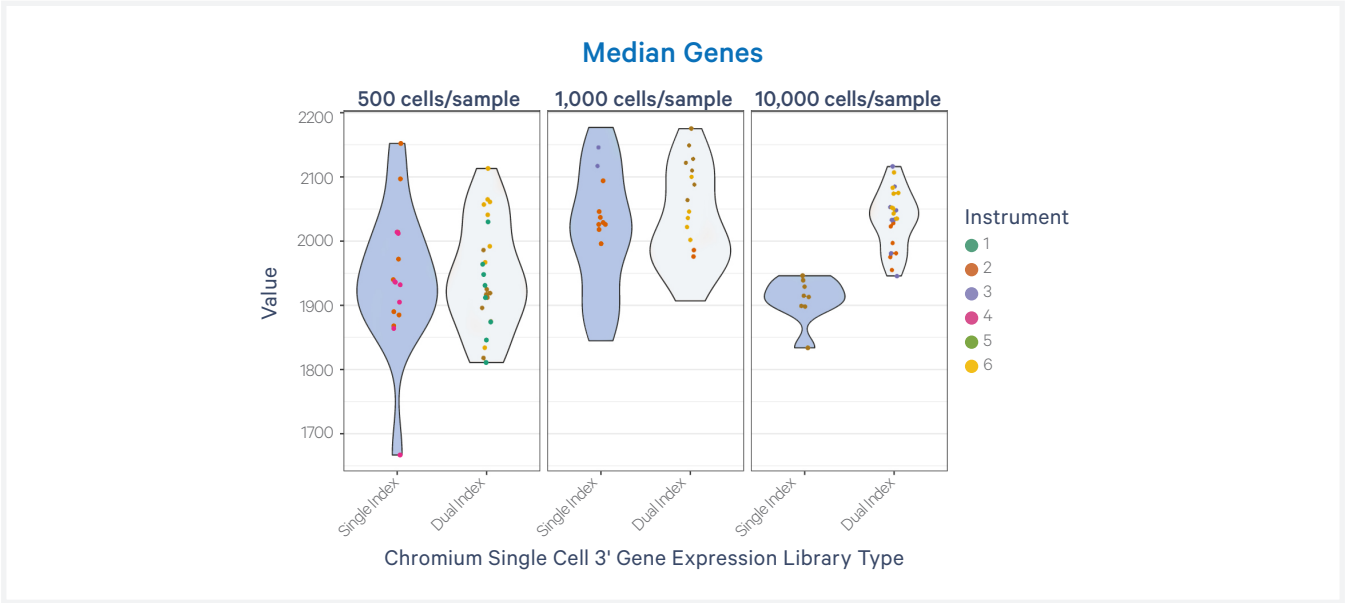

Figure 5. Data derived from Chromium Single Cell 3' Gene Expression dual and single index libraries generated using the Chromium Connect automated workflow (across multiple instruments) show comparable median genes per cell in data derived from PBMCs (targeting 500, 1,000 and 10,000 cells/sample).

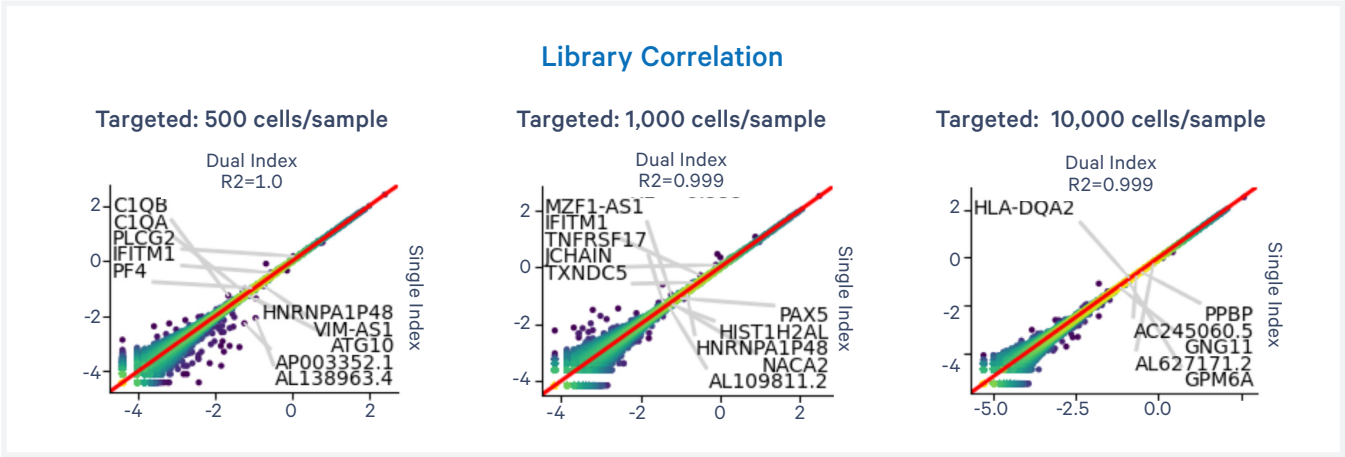

Figure 6. Comparable library chemistry correlation was observed between Chromium Single Cell 3' Gene Expression dual and single index libraries generated from PBMCs using the automated workflow as shown in the UMI count correlation plots for the indicated cell recovery.

References

This document provides guidance for the Chromium Connect: Single Cell 3' Gene Expression Dual Index Library Data Overview for use with:

- Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 User Guide (CG000286)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide (CG000315)
- Chromium Next GEM Single Cell 3' v3.1 Dual Index Libraries Technical Note (CG000325)

Document Revision Summary

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