TECHNICAL NOTE

Chromium Next GEM Single Cell 3' v3.1: Dual Index Libraries

Introduction

Chromium Single Cell Gene Expression Solution now enables generation of dual index libraries to study gene expression profiles, cell surface protein expression, and/or CRISPR screening in hundreds to thousands of cells. This Technical Note provides an overview of index hopping mitigation and highlights the key reagents and step overviews for generating dual index libraries, along with comparison of representative data derived from dual versus single index Chromium Single Cell 3' v3.1 libraries. Additionally, a detailed list of products and documents for generating dual index libraries is also provided.

Chromium Single Cell 3' Dual Index Libraries

Chromium Single Cell 3' libraries can now be generated with dual indices using Chromium Next GEM Single Cell 3' v3.1 reagents. These libraries are similar to previous Single Cell 3' v3.1 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, now 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' v3.1 (Dual Index) user guides.

Cell Ranger 4.0 analysis pipeline "cellranger mkfastq", used for Chromium Single Cell 3' libraries now supports demultiplexing of dual index libraries, ensuring that reads without a specified pair of dual indices are not assigned to a sample.

Index Hopping Mitigation

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 i7 index sequences (Figure 2). 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.

This document provides an overview of specific reagents used in the workflow to construct dual index libraries, along with library pooling recommendations, and sequencing configurations. Additionally, parity in results derived from dual index versus single index libraries is shown in Representative Data Highlights 1-3.

Figure 1. A representative Chromium Single Cell 3' dual index library schematic with sample index i5 and i7.

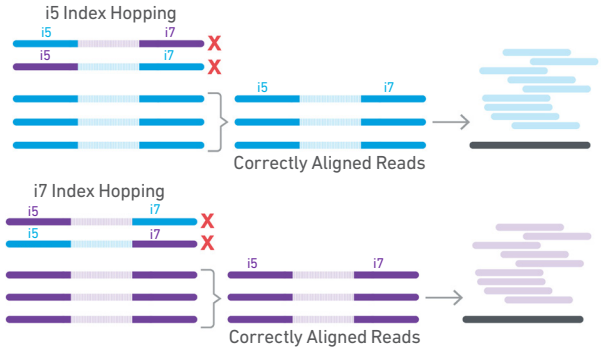


Figure 2. Removal of index-hopped reads during demultiplexing. Index-hopped reads are computationally filtered during demultiplexing, enabling correct alignment of sample-specific reads.

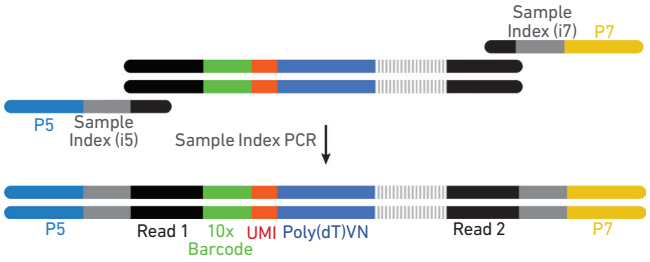
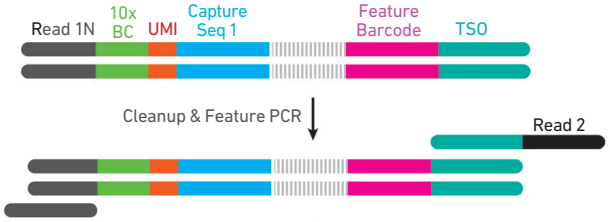
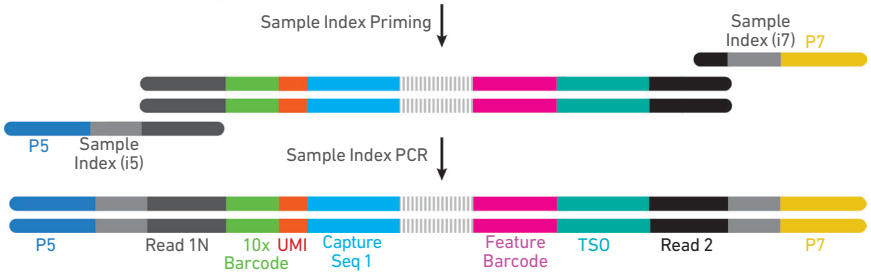
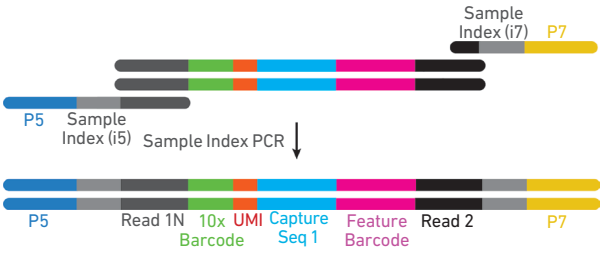
Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

Sample Index Reagents & Step Overview

DUAL INDEX

The Chromium Next GEM Single Cell 3' v3.1 workflow for generating dual index libraries is similar to the workflow for single index libraries, with a few specific reagent and protocol step updates. A "DUAL INDEX" icon is placed adjacent to the updated protocol steps in the relevant user guides. An illustrative overview of dual sample index addition steps with corresponding reagents/sample index kits along with the final library schematics is shown below.

Refer to Chromium Next GEM Single Cell 3' v3.1 – Product Lists & Documents (Dual Index) for detailed information regarding reagents and documents.

Reagents for Dual Indexing	Step Overview
Chromium Single Cell 3' Gene Expression Dual Index Library	
Dual Index Plate TT Set A (PN-1000215)	
Chromium Single Cell 3' CRISPR Screening Dual Index Library	
Feature SI Primers 3 (PN-2000263)	
Dual Index Kit NT Set A (PN-1000242)	
Chromium Single Cell 3' Dual Index Cell Surface Protein Dual Index Library	
Dual Index Kit NT Set A (PN-1000242)	

Library Pooling

Chromium Single Cell 3' v3.1 Gene Expression, CRISPR Screening, and/or Cell Surface Protein Dual Index libraries can be pooled together for sequencing. Given that the recommended sequencing read configurations differ between single index and dual index Chromium Single Cell 3' libraries, we recommend sequencing single index and dual index libraries separately. However, it may be possible to optimize pooling single and dual index libraries together for sequencing.

For more information, contact support@10xgenomics.com.

Sequencing

Sequencing Depth	
Gene Expression Dual Index Library	Minimum 20,000 read pairs per cell
CRISPR Screening Dual Index Library	Minimum 5,000 read pairs per cell (Minimum required Read 2 length is 70 bp)
Cell Surface Protein Dual Index Library	Minimum 5,000 read pairs per cell (Minimum required Read 2 length is 25 bp)
Sequencing Type	Paired-end, dual indexing
Sequencing Read	Recommended Number of Cycles
Read 1	28 cycles
i7 Index	10 cycles
i5 Index	10 cycles
Read 2	90 cycles

Representative Data

The Representative Data Highlights 1-3 provide a methods overview along with comparison of key results derived from various sample types, like Peripheral Blood Mononuclear Cells (PBMCs). The library types were generated using the specified Chromium Next GEM Single Cell 3' v3.1 reagents and protocols, were sequenced, and the data were analyzed and visualized as indicated in each of the Data Highlights.

The results shown in Figures 3-10 clearly demonstrate that both single and dual index versions of the Chromium Single Cell 3' v3.1 libraries yield comparable results in terms of cell clustering, proportion of cell subpopulations detected, library complexity, CRISPR sgRNA assignment and cell calling, and cell surface protein detection.

Representative Data Highlight 1

Gene expression - Overlapping cell populations & comparable library complexity for both chemistries

Methods Overview

	Gene Expression Library (Single Index)	Gene Expression Library (Dual Index)
Protocol	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (CG000204)	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 -Dual Index (CG000315)
Sequencing	Depth: ~100,000 read pairs per cell Cycles: Read 1–28; i7–8; Read 2–91	Depth: ~100,000 read pairs per cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90
Analysis **Visualization**	Cell Ranger 4.0 Loupe Browser 4.1	Cell Ranger 4.0 Loupe Browser 4.1

Results

Comparable cell clustering

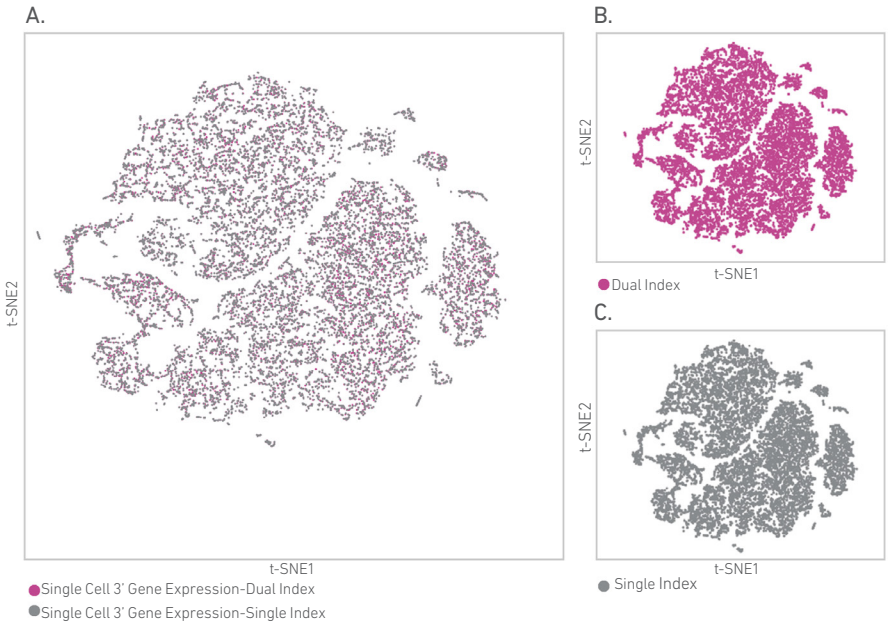


Figure 3. Chromium Single Cell 3' Gene Expression libraries generated from both chemistries show comparable clustering and overlapping cell populations. A. Aggregated analysis of both chemistries indicates minimal technical differences (Purple: Dual Index; Grey: Single Index). **B.** t-SNE plot generated from 10,915 PBMCs profiled using the Single Cell 3' Gene Expression v3.1 workflow (Dual Index). **C.** t-SNE plot generated from 10,958 PBMCs profiled using the Single Cell 3' Gene Expression v3.1 workflow (Single Index).

Contd.

Results

Same immune cell subpopulations

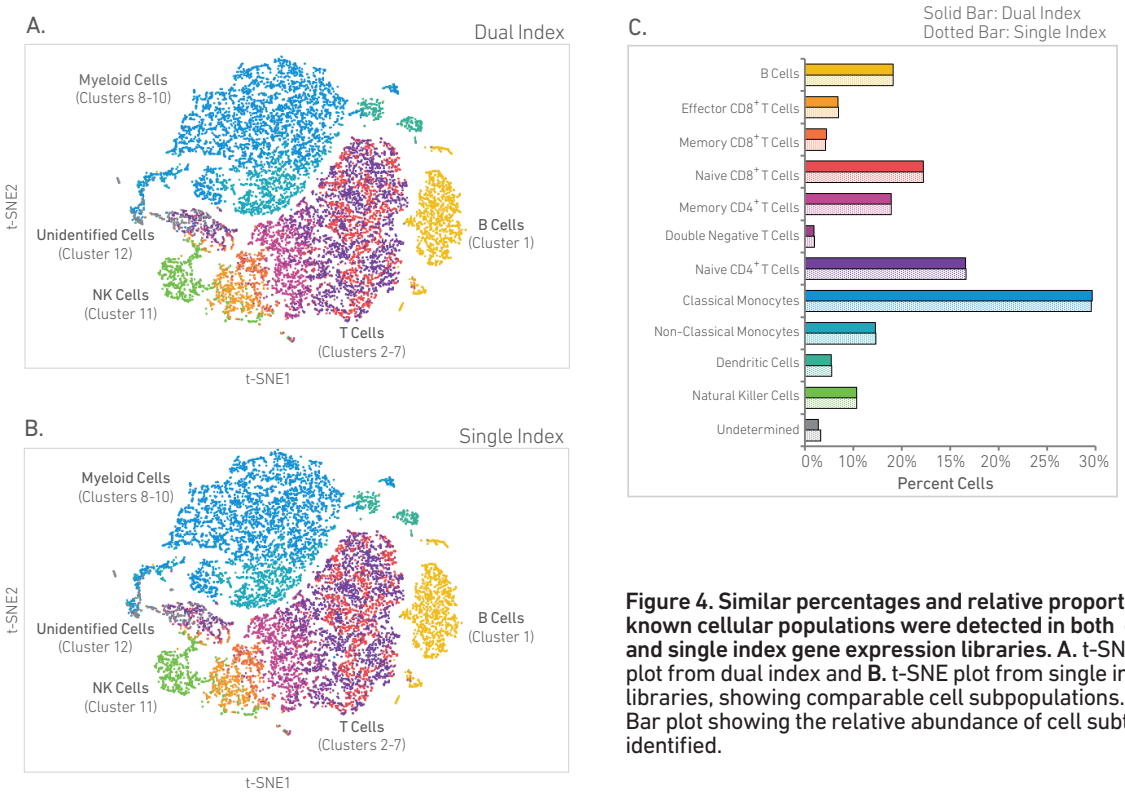


Figure 4. Similar percentages and relative proportions of known cellular populations were detected in both dual and single index gene expression libraries. A. t-SNE plot from dual index and **B.** t-SNE plot from single index libraries, showing comparable cell subpopulations. **C.** Bar plot showing the relative abundance of cell subtypes identified.

Comparable library complexity and correlation

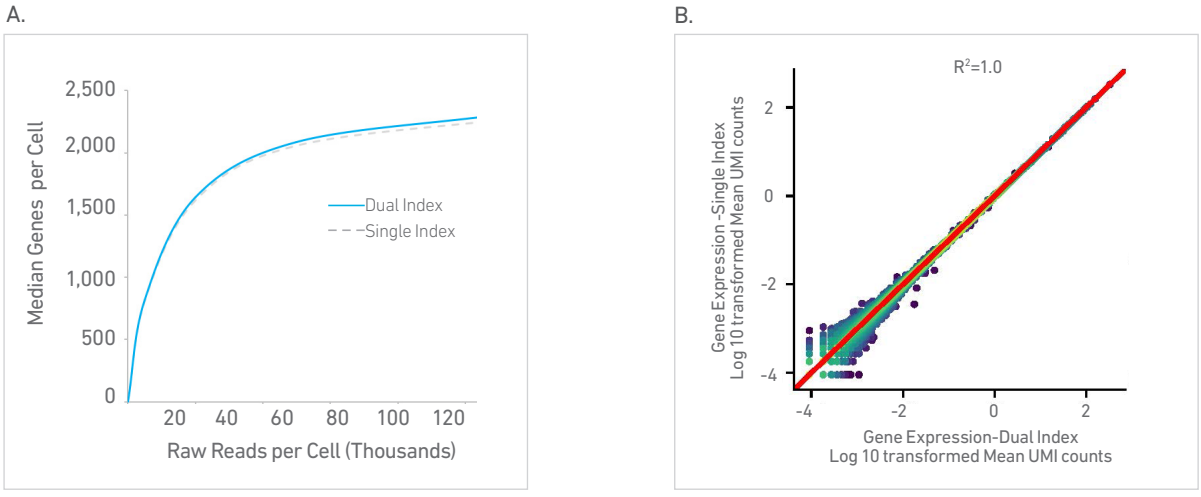
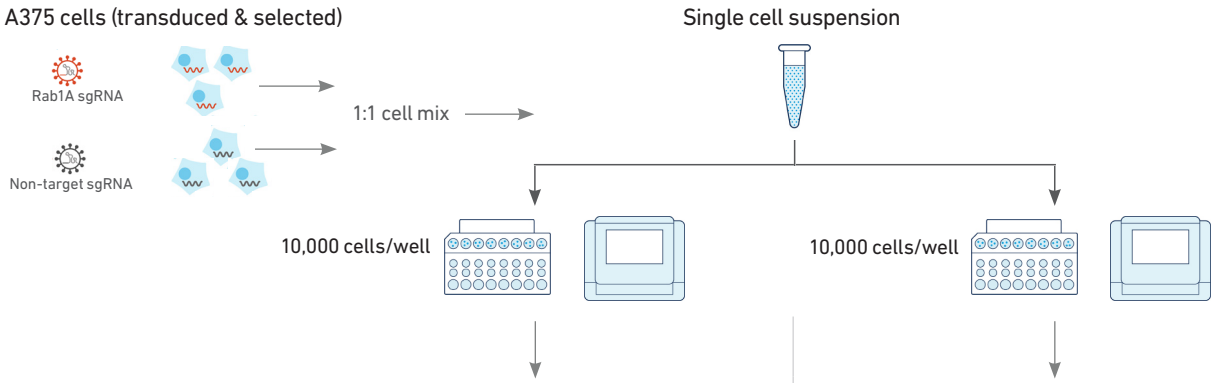


Figure 5. Comparable library complexity and chemistry correlation was observed between Chromium Single Cell 3' Gene Expression dual and single index libraries, as shown in the Median Genes per Cell (A) and UMI count correlation (B) plots.

Representative Data Highlight 2

CRISPR Screening - Comparable single guide RNA (sgRNA) assignment & cell calling

Methods Overview



	Gene Expression + CRISPR Libraries (Single Index)	Gene Expression + CRISPR Libraries (Dual Index)
Protocol	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcode-CRISPR (CG000205)	Chromium Next GEM Single Cell 3' Reagent Kits v3.1(Dual Index) with Feature Barcode-CRISPR (CG000316)
Sequencing	Depth: GEX-50,000, CRISPR-10,000 read pairs/cell Cycles: Read 1–28; i7–8; Read 2–91	Depth: GEX-50,000, CRISPR-10,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90
Analysis **Visualization**	Cell Ranger 4.0 Loupe Browser 4.1	Cell Ranger 4.0 Loupe Browser 4.1

Results

Comparable Rab1A sgRNA assignment

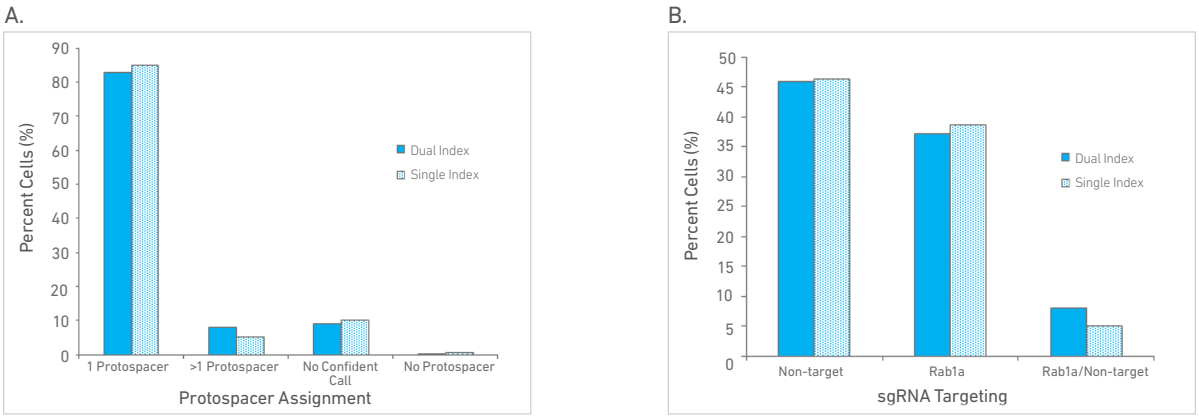


Figure 6. Comparable summary (A) and specific sgRNA (B) assignment was observed in data from Chromium Single Cell 3' CRISPR Screening dual and single index libraries generated from a 1:1 mix of A375 cells that had been transduced with either Rab1a sgRNA or non-targeting sgRNA.

Contd.

Results

Comparable sgRNA calling

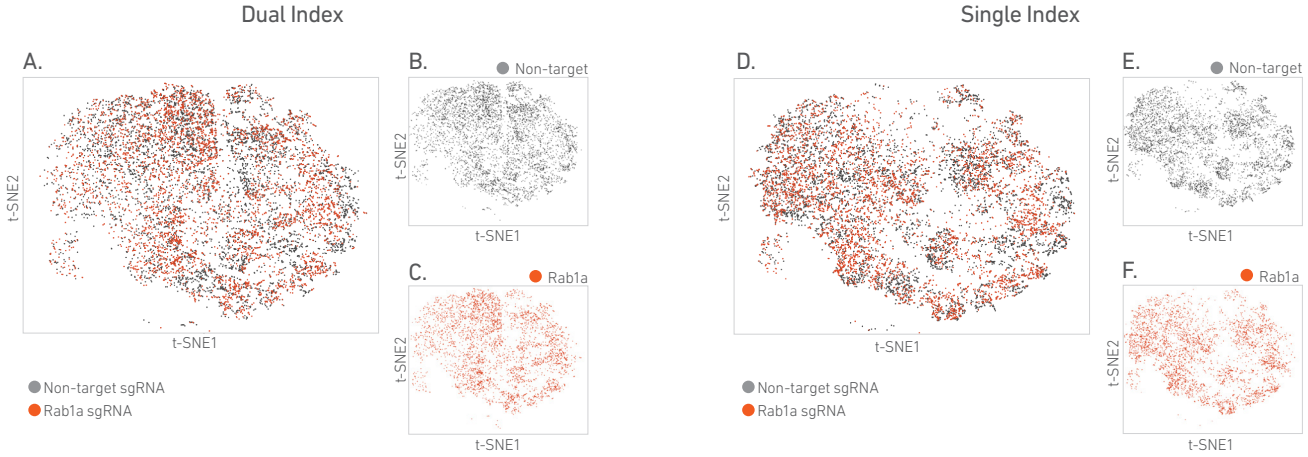


Figure 7. Chromium Single Cell 3' CRISPR Screening libraries generated from both chemistries show comparable sgRNA calling. The calls were generated by Cell Ranger 4.0 and then visualized in Loupe Browser. Dual index data showing (Grey: Non-target; Red: Rab1a) aggregated analysis (A), along with individual t-SNE plots calling 5,396 non-target sgRNA (B) and 4,369 Rab1a sgRNA (C) cells. Single index data showing (Grey: Non-target; Red: Rab1a) aggregated analysis (D), along with t-SNE plots calling 5,287 non-target sgRNA (E) and 4,416 Rab1a sgRNA (F) cells.

Comparable Rab1a knockdown

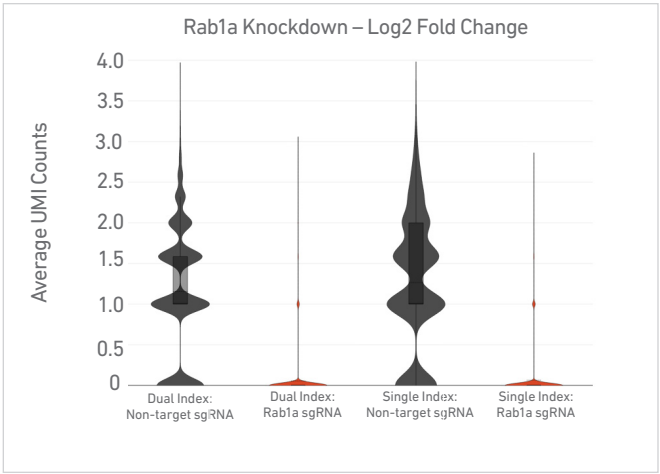
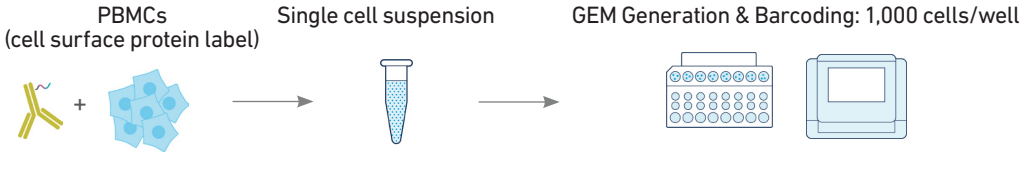


Figure 8. Comparable Rab1a knockdown was observed in data from Chromium Single Cell 3' CRISPR Screening libraries generated from both chemistries.

Representative Data Highlight 3

Cell Surface Protein - Comparable cell subpopulation expression

Methods Overview



cDNA Generation & Amplification

	GEX + Cell Surface Protein Libraries (Single Index)	GEX + Cell Surface Protein Libraries (Dual Index)
Protocol	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcode-Cell Surface Protein (CG000207)	Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode-Cell Surface Protein (CG000317)
Sequencing	Depth: GEX-50,000, Protein-10,000 read pairs/cell Cycles: Read 1–28; i7–8; Read 2–91	Depth: GEX-50,000, Protein-10,000 read pairs/cell Cycles: Read 1–28; i5–10; i7–10; Read 2–90
Analysis	Cell Ranger 4.0 Loupe Browser 4.1	Cell Ranger 4.0 Loupe Browser 4.1

Results

UMI Count Comparison

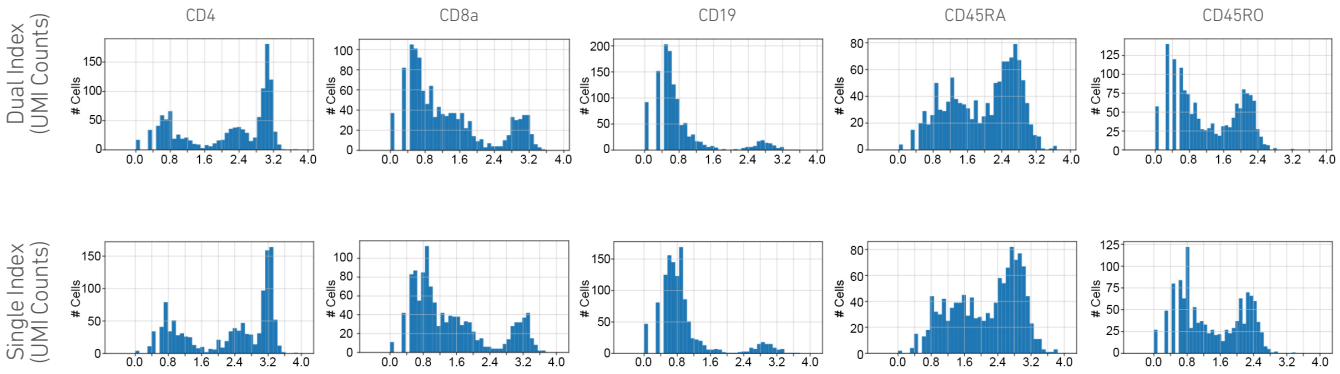


Figure 9. Comparable cell populations were observed when comparing UMI counts derived from Chromium Single Cell 3' Cell Surface Protein dual index (top panel) and single index (bottom panel) libraries.

Contd.

Representative Data Highlight 3 Contd.

Results

Comparable cell populations

Figure 10. Chromium Single Cell 3' Gene Expression and Cell Surface Protein libraries generated from both chemistries show comparable clustering and overlapping populations of cells. Cell surface protein (A) and gene expression (C) t-SNE plots generated from 1,196 PBMCs profiled using the Single Cell 3' v3.1 workflow (Dual Index). Cell surface protein (B) and gene expression (D) t-SNE plots generated from 1,205 PBMCs profiled using the Single Cell 3' Gene Expression v3.1 workflow (Single Index).

Chromium Next GEM Single Cell 3' v3.1 – Product List & Documents (Dual Index)

Product list for generating Chromium Single Cell 3' Gene Expression Dual Index Libraries:

REAGENT KITS	REACTIONS	PART NUMBER (PN)
Chromium Next GEM Single Cell 3' Kit v3.1	16 rxns 4 rxns	1000268 1000269
Chromium Next GEM Chip G Single Cell Kit	48 rxns 16 rxns	1000120 1000127
Dual Index Kit TT Set A	96 rxns	1000215
INSTRUMENT		
Chromium Controller & Next GEM Accessory Kit	-	120223 (12 month warranty) 120246 (24 month warranty)
SOFTWARE		
Cell Ranger Analysis Pipeline (DOWNLOAD)		
Loupe Browser (DOWNLOAD)		
DOCUMENTS (for Single Cell 3' Gene Expression Dual Index Libraries ONLY)		
User Guide: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) (CG000315)		

If using Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) protocols with Feature Barcode technology, the 3' Feature Barcode Library Kit is required in addition to all the products listed above. Refer to the indicated documents for specific guidance.

REAGENT KITS	REACTIONS	PART NUMBER (PN)
3' Feature Barcode Library Kit	16 rxns	1000276
Dual Index Kit NT Set A	96 rxns	1000242
DOCUMENTS (for Single Cell 3' Gene Expression + Single Cell 3' CRISPR Screening Dual Index Libraries ONLY)		
User Guide: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening (CG000316) Tech Note : Guide RNA Specifications Compatible with Feature Barcoding Technology for CRISPR Screening (CG000197)		
DOCUMENTS (for Single Cell 3' Gene Expression + Single Cell 3' Cell Surface Protein Dual Index Libraries ONLY)		
User Guide: Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcoding technology for Cell Surface Protein (CG000317) Demonstrated Protocol : Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (CG000149)		

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