Chromium Connect:
Modular Gene Expression Workflow & Data Overview

Introduction

Chromium Connect now offers modular workflow options along with the previously available continuous workflow for automated generation of Chromium Next GEM Single Cell Gene Expression libraries. The modular workflow allows greater flexibility by enabling the use of cDNA generated using an automated workflow followed by automated library construction. Additionally, a blended workflow in which the cDNA is generated using a compatible 10x Genomics manual assay can be used for automated preparation of sequencing-ready, single cell gene expression libraries, minimizing technical variation in data. This Technical Note provides an overview of the Chromium Connect continuous and modular workflow options and highlights consistency and reproducibility in Chromium Single Cell Gene Expression data derived using these workflows.
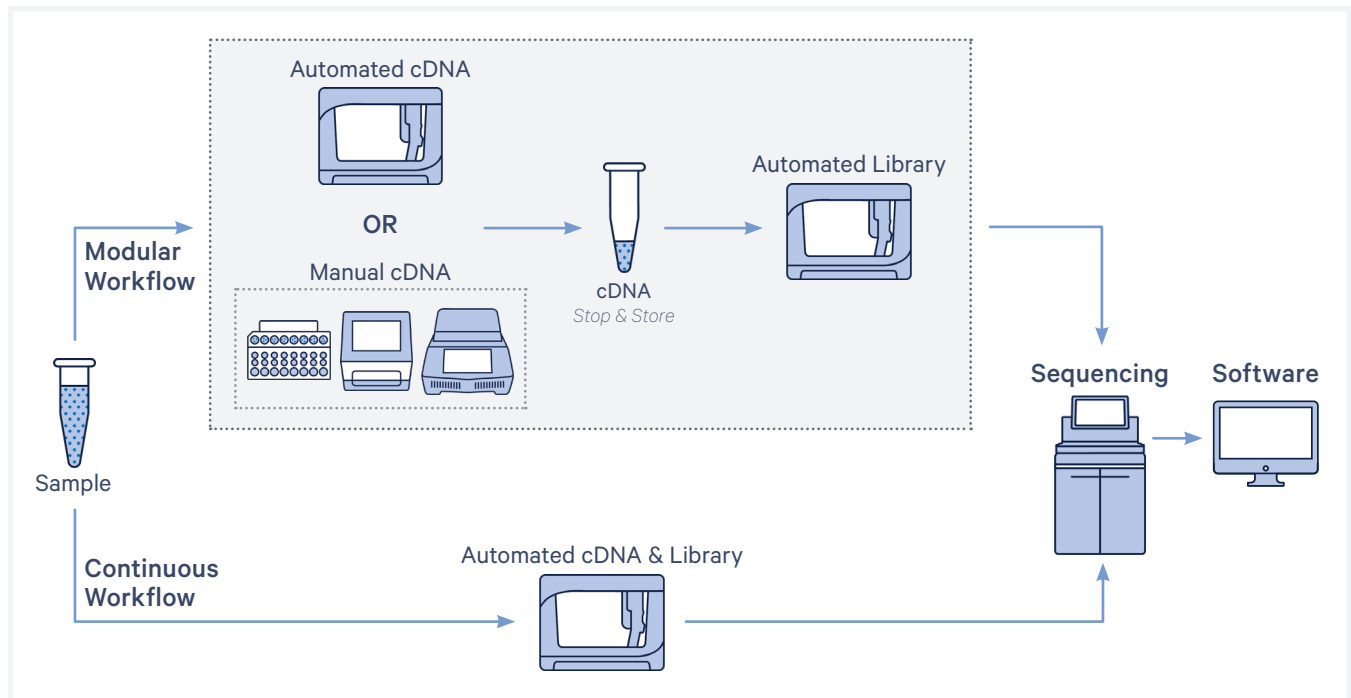

Figure 1. Overview of Chromium Connect continuous and modular workflows for generating Chromium Next GEM Single Cell Gene Expression libraries.

The key differences between the Chromium Next GEM Automated Single Cell continuous and modular workflows are presented below (Table 1). Refer to the relevant user guides for complete information.

	Chromium Next GEM Automated Single Cell	
	Continuous Workflow	**Modular Workflow**
Sample Prep	Recommendations for preparing single cell suspensions are the same for the continuous and modular workflows. Visit the 10x Genomics Support website for sample preparation specifics.	
10x Genomics Reagents*	Chromium Next GEM Automated Single Cell Kit (4 or 24 rxns) Chromium Next GEM Automated Chip Kit (16 or 48 rxns) - Index Kit (96 rxns)	Chromium Next GEM Automated Single Cell cDNA Kit (24 rxns) Chromium Next GEM Automated Chip Kit (16 or 48 rxns) Chromium Next GEM Automated Single Cell Library Construction Kit (24 rxns) Index Kit (96 rxns)
cDNA & Library Construction*	Sample to sequencing-ready libraries on Chromium Connect using the automated continuous workflow	Step 1: Generate cDNA Sample to cDNA generation on Chromium Connect using the automated modular workflow OR Sample to GEM & cDNA generation manually using a compatible 10x Genomics manual workflow* Stop & store cDNA Step 2: Automated library construction cDNA to sequencing-ready libraries on Chromium Connect using the automated modular workflow
Library	The single cell gene expression libraries generated using the continuous and modular workflows have the same configuration. A schematic of the Single Cell 3' v3.1 Gene Expression dual index library that may be generated using either the continuous or the modular workflow is shown below. 	
Sequencing	The sequencing recommendations for Single Cell Gene Expression dual index libraries are the same irrespective of the workflow used to generate the libraries.	
Software	Sequencing data derived from libraries generated using either protocol can be analyzed and visualized using the latest versions of Cell Ranger and Loupe Browser available on the 10x Genomics Support website.	

*See *Product List & Documents* section and *Compatible 10x Genomics Assays* (Table 2) for more information

Table 1. Chromium Connect Next GEM Automated Gene Expression continuous and modular workflow comparison.

Manual Workflows Automated Library Construction

Table 2 shows 10x Genomics manual cDNA generation workflows that are supported for automated gene expression library construction on Chromium Connect. Currently, automated gene expression libraries can be generated from cDNA input derived from Chromium Next GEM Single Cell 3' v3.1 standard and low throughput LT workflows (high throughput HT coming soon) and Single Cell 5' v2 standard workflow (high throughput HT coming soon). Additional single cell manual assays will also be supported soon, enabling automated generation of sequencing-ready libraries from multiple manual assays.

10x Genomics Assay	Manual Workflow Reagents (cDNA generation)	cDNA Compatible with Automated Gene Expression Library Construction
Single Cell 3' v3.1 Gene Expression	Chromium Next GEM Single Cell 3' Reagent Kits v3.1	Yes
	Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (low throughput)	Yes
	Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (high throughput)	*Coming soon*
Single Cell 5' v2 Gene Expression	Chromium Next GEM Single Cell 5' Reagent Kits v2	Yes
	Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (high throughput)	*Coming soon*

Table 2. 10x Genomics single cell manual assays that are supported for automated gene expression library construction on Chromium Connect.

Data Highlight 1

Methods Overview

Chromium Single Cell 3' v3.1 Gene Expression libraries (from human HEK293T and mouse NIH/3T3 cells mixed 1:1; targeting 10,000 cells) and Chromium Single Cell 5' v2 Gene Expression libraries (from human PBMCs targeting 10,000 cells) were generated using the Chromium Connect Automated continuous (sample to automated library construction) and modular workflows (sample to automated cDNA generation followed by automated library construction).

Cells from the same aliquot were used for both the continuous and modular workflows. For Single Cell 3' v3.1, two Chromium Connect instruments were used for the continuous workflow and three instruments were used for the modular workflow. For Single Cell 5' v2, three Chromium Connect instruments were used for each of the two workflows. Each instrument processed 8 replicates per run. The libraries were sequenced and the data were analyzed as described in the respective user guides and the 10x Genomics Support website (see References).

Results

The results shown in Table 3 demonstrate that the automated continuous and automated modular workflows perform comparably in Single Cell 3' v3.1 and Single Cell 5' v2 Gene Expression library construction. Concordant cell type identification was also observed in Single Cell 5' v2 Gene Expression data (Figure 2) derived using both workflows and in Single Cell 3' v3.1 Gene Expression data (not shown).

Library Metrics	Chromium Next GEM Automated			
	Single Cell 3' v3.1 (293T+3T3 – 10,000 cells)		Single Cell 5' v2 (hPBMCs – 10,000 cells)	
	Continuous Workflow	Modular Workflow	Continuous Workflow	Modular Workflow
Estimated number of cells	8,897	9,075	8,665	8,540
Fraction reads in cells	87%	88%	95%	95%
Valid barcodes	98%	98%	92%	92%
Valid UMIs	100%	100%	100%	100%
Fraction reads usable	52%	55%	54%	56%
Reads mapped confidently to transcriptome	61%	64%	59%	60%
Reads mapped confidently to genome	91%	93%	78%	80%
Reads mapped confidently to intronic regions	22%	22%	11%	11%
Reads mapped confidently to exonic regions	64%	67%	64%	66%
Fraction reads unmapped	4%	3%	7%	5%
Reads with full poly A sequence	2%	1%	5%	4%
Reads with full template switch oligo sequence	6%	5%	0%	0%
Median genes per cell (50,000 raw read pairs/cell)	4,741	4,846	2,153	2,112
Median UMI counts (50,000 raw read pairs/cell)	19,046	19,580	5,764	5,478

Table 3. Comparable Chromium Next GEM Single Cell 3' v3.1 and 5' v2 Gene Expression library metrics generated using either the Chromium Connect Next GEM Automated Gene Expression continuous or modular workflow. For Single Cell 3' v3.1, two Chromium Connect instruments were used for the continuous workflow and three instruments were used for the modular workflow. For Single Cell 5' v2, three Chromium Connect instruments were used for each of the two workflows. 8 replicates were processed per run.
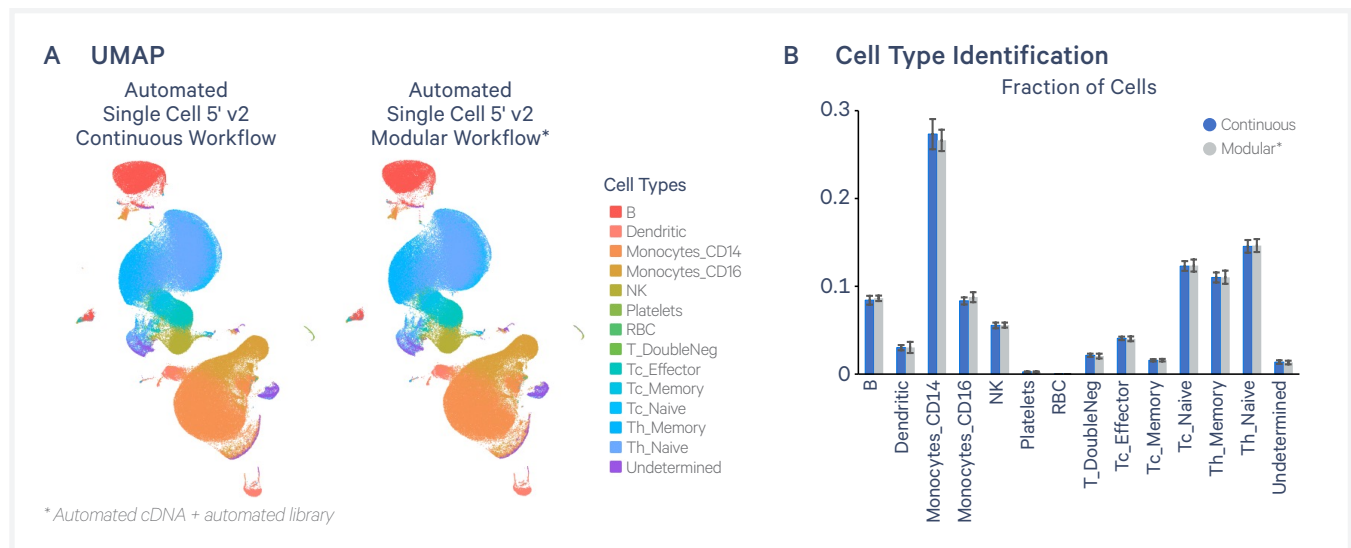
Comparable Cell Type Identification

Figure 2. The gene expression profiles derived from Chromium Single Cell 5' v2 Gene Expression libraries generated using either the Chromium Connect Next GEM Automated continuous or modular workflow (three instruments for each workflow; 8 replicates/instrument) identify the same major cell types in human PBMCs. The gene expression data from the workflows show similar cell clustering (A) and comparable cell type identification (B). (Data not shown - comparable results were observed for Single Cell 3' v3.1 Gene Expression data derived using the two workflows).

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Data Highlight 2

Methods Overview

Human PBMCs were used to generate cDNA using Chromium Controller following the Single Cell 3' v3.1 and 5' v2 Gene Expression manual workflows. The manually generated cDNA was used as input for manual and automated generation of Chromium Single Cell 3' v3.1 or 5' v2 Gene Expression libraries (targeting 1,200 and 5,000 cells respectively) for side-by-side data comparison. For Single Cell 3' v3.1, two users performed the manual workflow and three Chromium Connect instruments were used for the modular workflow. For Single Cell 5' v2, one user

performed the manual workflow and three Chromium Connect instruments were used for the modular workflow. Each user and each instrument processed 8 replicates per run. The libraries were sequenced and the data were analyzed as described in the respective user guides and the 10x Genomics Support website (see References).

Results

The data shown in Figure 3 demonstrates that the manual and the modular workflows capture comparable library complexity for Single Cell 3' v3.1 and Single Cell 5' v2 Gene Expression.

Comparable Library Complexity
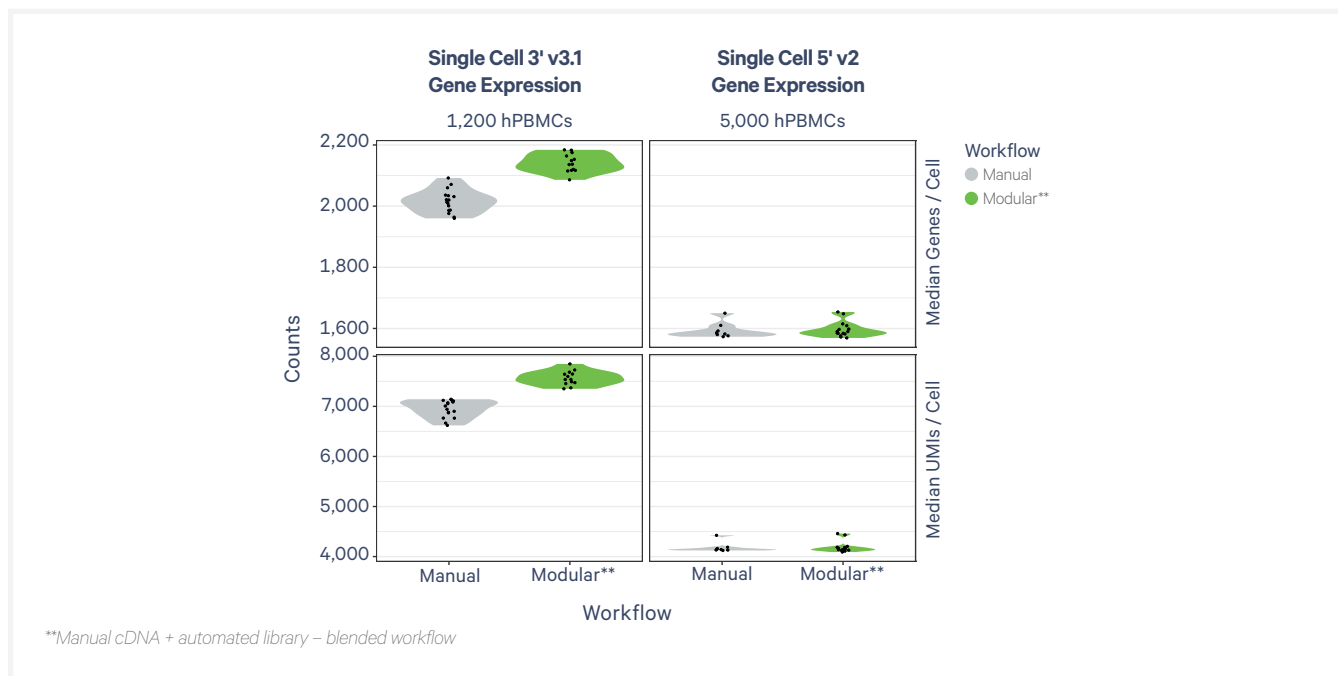
Figure 3. Chromium Single Cell Gene Expression libraries constructed using either the manual workflow (Single Cell 3' - two users; Single Cell 5' - one user; 8 replicates/user) or the modular workflow (manual cDNA + automated library blended workflow; Single Cell 3' - three instruments; Single Cell 5' - two instruments; 8 replicates/instrument) show comparable library complexity and sensitivity for Single Cell 3' v3.1 and 5' v2 Gene Expression data. Similar median genes per cell (top panels) and median UMI counts per cell (bottom panels) were observed. The Single Cell 3' v3.1 and 5' v2 Gene Expression datasets shown were down sampled to 50,000 and 20,000 raw read pairs per cell respectively.

Chromium Next GEM Automated Single Cell Gene Expression – Product List & Documents

Product list for generating Chromium **Single Cell 3'** Gene Expression Libraries using Chromium Connect			
SC3' Workflows	**Reagent Kits**	**Reactions**	**Part Number**
Automated Continuous (sample to automated library)	Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1	4 rxns 24 rxns	1000147 1000141
	Chromium Next GEM Chip G Automated Single Cell Kit	16 rxns 48 rxns	1000146 1000136
	Dual Index Kit TT Set A or Single Index Kit T Set A	96 rxns 96 rxns	1000215 1000213
Modular - Automated cDNA + Automated Library	cDNA Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1	24 rxns	1000424
	Chromium Next GEM Chip G Automated Single Cell Kit	16 rxns 48 rxns	1000146 1000136
	Library Automated Library Construction Kit	24 rxns	1000428
	Dual Index Kit TT Set A	96 rxns	1000215
Modular - Manual cDNA* + Automated Library	cDNA cDNA generated manually using compatible 10x Genomics assays*	-	-
	Library Automated Library Construction Kit	24 rxns	1000428
	Dual Index Kit TT Set A	96 rxns	1000215
Software	Cell Ranger Analysis Pipeline (DOWNLOAD)		
	Loupe Browser (DOWNLOAD)		
Automated Assay Documents	Chromium Next GEM Automated Single Cell 3' Reagent Kits User Guide	CG000286	
	Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1 Supplemental User Guide	CG000472	
	Automated Gene Expression Library Construction User Guide	CG000474	
	Refer to 10x Genomics Support website for additional documents, including Chromium Connect Calculators.		

cDNA may be generated using compatible manual workflows (standard or low throughput) for single cell gene expression on Chromium X/iX or Chromium Controller. Refer to the 10x Genomics website for the most current information regarding all supported assays.

Product list for generating Chromium **Single Cell 5'** Gene Expression Libraries using Chromium Connect				
SC5' Workflows		**Reagent Kits**	**Reactions**	**Part Number**
Automated Continuous (sample to automated library)		Chromium Next GEM Automated Single Cell 5' Kit v2	4 rxns 24 rxns	1000298 1000290
		Chromium Next GEM Chip K Automated Single Cell Kit	16 rxns 48 rxns	1000297 1000289
		Dual Index Kit TT Set A	96 rxns	1000215
Modular - Automated cDNA + Automated Library	cDNA	Chromium Next GEM Automated Single Cell 5' cDNA Kit v2	24 rxns	1000425
		Chromium Next GEM Chip K Automated Single Cell Kit	16 rxns 48 rxns	1000297 1000289
	Library	Automated Library Construction Kit	24 rxns	1000428
		Dual Index Kit TT Set A	96 rxns	1000215
Modular - Manual cDNA* + Automated Library	cDNA	cDNA generated manually using compatible 10x Genomics assays*	-	-
	Library	Automated Library Construction Kit	24 rxns	1000428
		Dual Index Kit TT Set A	96 rxns	1000215
Software		Cell Ranger Analysis Pipeline (DOWNLOAD)		
		Loupe Browser (DOWNLOAD)		
Automated Assay Documents		Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 User Guide	CG000384	
		Chromium Next GEM Automated Single Cell 5' cDNA Kit v2 Supplemental User Guide	CG000473	
		Automated Gene Expression Library Construction User Guide	CG000474	
		Refer to 10x Genomics Support website for additional documents, including Chromium Connect Calculators.		

cDNA may be generated using compatible manual workflows (standard or low throughput) for single cell gene expression on Chromium X/iX or Chromium Controller. Refer to the 10x Genomics website for the most current information regarding all supported assays.

References

1. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide (CG000315)

2. Chromium Next GEM Automated Single Cell 3' Reagent Kits User Guide (CG000286)

3. Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1 Supplemental User Guide (CG000472)

4. Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) User Guide (CG000331)

5. Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 User Guide (CG000384)

6. Chromium Next GEM Automated Single Cell 5' cDNA Kit v2 Supplemental User Guide (CG000473)

7. Automated Gene Expression Library Construction User Guide (CG000474)

Document Revision Summary

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