

SUPPLEMENTAL USER GUIDE

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2

FOR USE WITH

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2, 24 rxns PN-1000425

PAIRS WITH DOCUMENTS

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

Chromium Connect Instrument User Guide (CG000180)

Automated Gene Expression Library Construction User Guide (CG000474)

[For Chromium Next GEM Automated Single Cell 5' v2 with Feature Barcode technology for Cell Surface Protein/Immune Receptor Mapping](#)

Use the Chromium Automated Single Cell 5' Feature Barcode Library Construction Kit, 24 rxns PN-1000455. Refer to the Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (CG000507).

Notices

Document Number

CG000473 • Rev C

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Document Revision Summary

Document Number	CG000473
Title	Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2 Supplemental User Guide
Revision	Rev B to C
Revision Date	April 2023
Specific Changes	<ul style="list-style-type: none">• Updated to include Automated Library Construction Kit, 4 rxns PN-1000429 (page 5)• Revised to include the updated cDNA Generation Kit name (no change in kit configuration, kit components, reagent compositions, and part numbers; page 6)
General Changes	<ul style="list-style-type: none">• Updated for general minor consistency of language and terms throughout

Automated Modular Workflow

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2 Workflow Overview

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2

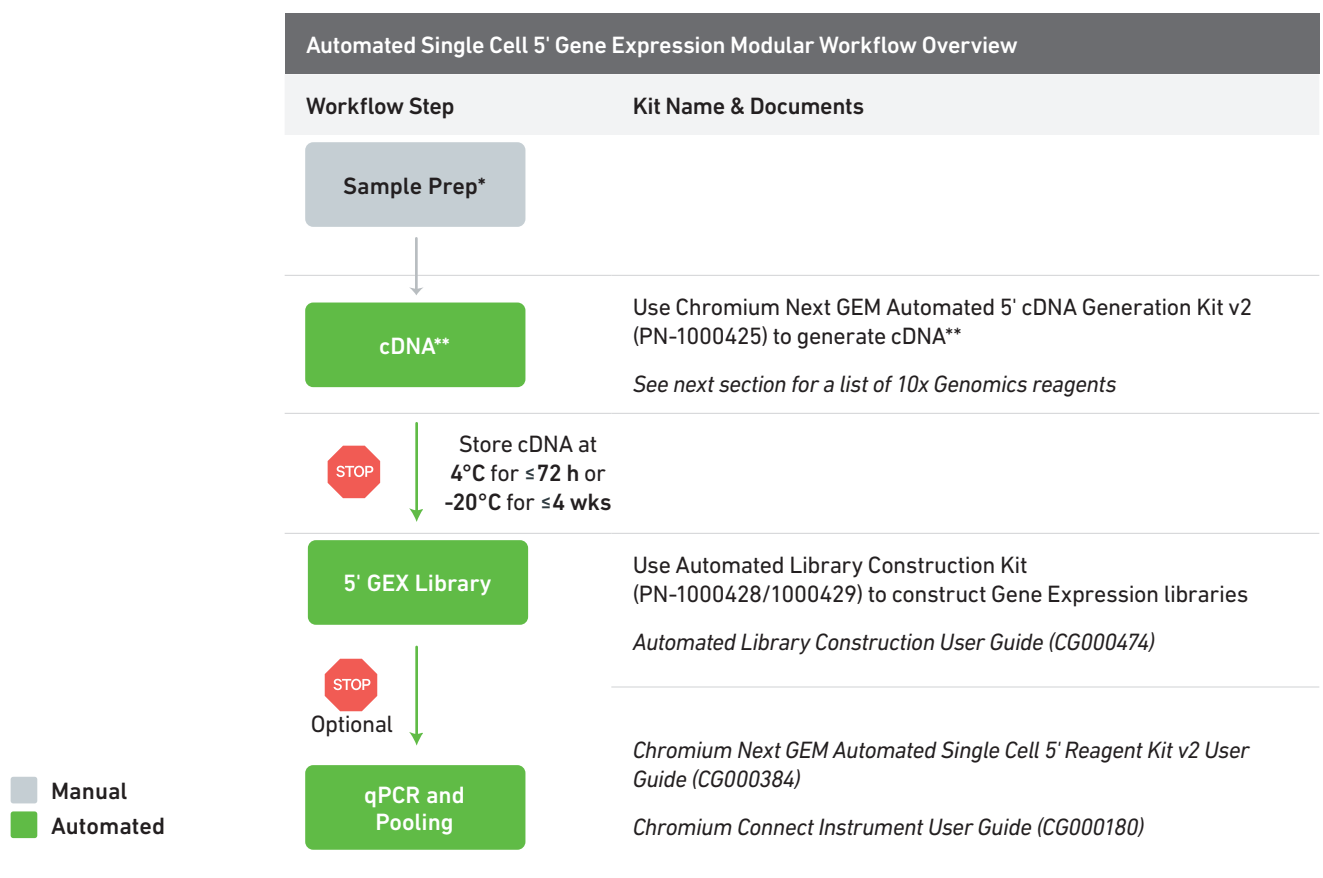
Protocol Steps & Timing

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2 Workflow Overview

This is a supplemental document that provides an overview of the Chromium Next GEM Automated Single Cell 5' Gene Expression modular workflow along with a list of 10x Genomics reagents needed to generate cDNA from single cell suspension. As part of this automated flexible workflow, cDNA** can be generated using the Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2 (PN-1000425). The cDNA can be stored at 4°C or -20°C or used directly for Single Cell 5' Library Construction using the Automated Library Construction Kit (PN-1000428/1000429) as described in the Automated Gene Expression Library Construction User Guide (CG000474).

For information on sample preparation, carrier loading and protocol guidelines, refer to the Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 User Guide (CG000384) and the instrument user interface.

For instrument operation, refer to the Chromium Connect Instrument User Guide (CG000180).



If assessing Cell Surface Protein Expression/Immune Receptor Mapping:

**Ensure that the samples are labeled as per Demonstrated Protocols CG000149 or CG000203*

***To simultaneously generate cDNA from mRNA and DNA from the Feature Barcode labeling the Cell Surface Protein, use Feature cDNA Primers 4 (PN-2000277) included in the Automated 5' Feature Barcode Primer Kit v2 (PN-1000454). Refer to the Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (CG000507) for more information.*

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2

All reagent tube strips & tubes are for one time use only. DO NOT reuse.

Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2, 24 rxns PN-1000425

cDNA Generation Kit & cDNA Kit are the same kits (no difference in kit configuration, components, reagent compositions, and part numbers).

Reagent volumes and colors are different in each of the module types, not all module tubes contain reagents.

Chromium Next GEM Automated Single Cell Kit, cDNA Generation Module 1, 24 rxns PN-1000395 (store at 4°C)

Chromium

Next GEM Automated Single Cell Kit,
cDNA Generation Module 1, 24 rxns

<input checked="" type="checkbox"/>	Single Cell cDNA Generation Module 1	24 tube strips
<input type="checkbox"/>	Dynabeads™ MyOne™ SILANE	6 tubes (PN-2000048)

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cDNA Generation Module 1



Chromium Next GEM Automated Single Cell Kit, cDNA Generation Module 2, 24 rxns PN-1000396 (store at -20°C)

Chromium

Next GEM Automated Single Cell Kit,
cDNA Generation Module 2, 24 rxns

<input checked="" type="checkbox"/>	Single Cell cDNA Generation Module 2	24 tube strips
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cDNA Generation Module 2



Chromium Next GEM Automated Single Cell 5' Kit v2, cDNA Generation Module 3, 24 rxns PN-1000398 (store at -20°C)

Chromium

Next GEM Automated Single Cell 5' Kit v2,
cDNA Generation Module 3, 24 rxns

<input type="checkbox"/>	Single Cell cDNA Generation Module 3	24 tube strips
	Poly-dT RT Primer	6 tubes (PN-2000007)

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cDNA Generation Module 3



Chromium Next GEM Automated Single Cell 5' cDNA Generation Kit v2, 24 rxns PN-1000425

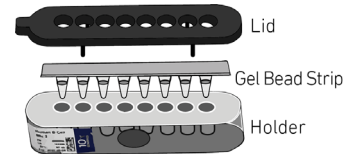
**Chromium Next GEM Automated Single Cell 5' Gel Bead Kit v2,
24 rxns PN-1000291 (store at -80°C)**

Chromium
Next GEM Automated
Single Cell 5' Gel Bead Kit v2, 24 rxns

Single Cell 5' v2 Gel Beads 3 tube strips

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Chromium Next GEM Chip K Automated Single Cell Kit, 48 rxns PN-1000289 (store at ambient temperature)

Chromium Partitioning Oil			Chromium 50% Glycerol		
	#	PN		#	PN
<input checked="" type="radio"/> Partitioning Oil	6	2000190	<input type="radio"/> 50% Glycerol	6	2000109

Chromium Next GEM Chip K Automated Single Cell		
	#	PN
Next GEM Chip K Automated Single Cell (gasket attached)	6	2000371

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Chromium Next GEM Chip K Automated Single Cell Kit, 16 rxns PN-1000297 (store at ambient temperature)

Chromium Partitioning Oil			Chromium 50% Glycerol		
	#	PN		#	PN
<input checked="" type="radio"/> Partitioning Oil	2	2000190	<input type="radio"/> 50% Glycerol	2	2000109

Chromium Next GEM Chip K Automated Single Cell		
	#	PN
Next GEM Chip K Automated Single Cell (gasket attached)	2	2000371

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Use the 10x Genomics reagents listed above for automated cDNA* generation. After cDNA generation, store cDNA until ready to proceed to Gene Expression Library Construction using the Automated Library Construction Kit (PN-1000428/1000429). Refer to the Automated Gene Expression Library Construction User Guide (CG000474) for details.

*For Chromium Next GEM Automated Single Cell 5' v2 with Feature Barcode technology for Cell Surface Protein/Immune Receptor Mapping, use the Chromium Automated Single Cell 5' Feature Barcode Library Construction Kit (PN-1000455). Refer to the Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (CG000507) for details.

Protocol Steps & Timing

The table below provides an overview of the complete automated modular workflow steps and timing

Steps	Timing
Manual	
Cell Preparation (Dependent on Cell Type)	~1-1.5 h
Gather & Load Reagents and Consumables	~60 min
Automated Single Cell Gene Expression cDNA	
Master Mix Preparation	
Chromium Automated Controller Loading	
GEM Generation	
OPTIONAL Confirm GEM Generation (Manual, 5 min)	
Post GEM RT-Cleanup – Dynabead	~4.5 h Walk-away time
cDNA Amplification*	
cDNA Cleanup – SPRIselect	
STOP Stop after SPRI clean up, store cDNA at 4°C for ≤72 hrs or -20°C for ≤4 wks cDNA QC & Quantification (Manual, 50 min; best practice)	
Automated Single Cell Gene Expression Library	
Fragmentation, End Repair & A-tailing	
Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	~4 h Walk-away time
Adaptor Ligation	
Post Ligation Cleanup- SPRIselect	
Sample Index PCR	
Post Sample Index PCR Double Sided Size Selection- SPRIselect	
Manual	
Post Library Construction QC	50 min
OPTIONAL Library Quantification qPCR & Library Pooling	

*For Chromium Next GEM Automated Single Cell 5' v2 with Feature Barcode technology, cDNA from mRNA and DNA from the Feature Barcode will be generated simultaneously. Remove the supernatant from the instrument deck and store at 4°C or at -20°C for up to 5 weeks before proceeding to the next automated step (Feature Barcode DNA Supernatant – SPRIselect Cleanup). Refer to the Chromium Next GEM Automated Single Cell 5' Reagent Kits v2 with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (CG000507) for details.