

CG000472 Rev A



SUPPLEMENTAL USER GUIDE

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1



FOR USE WITH

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1, 24 rxns PN-1000424

PAIRS WITH DOCUMENTS

Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 User Guide (CG000286)

Chromium Connect Instrument User Guide (CG000180)

Automated Library Construction User Guide (CG000474)

Notices

Document Number

CG000472 • Rev A

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**Document
Revision
Summary**

Document Number	CG000472
Title	Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1 Supplemental User Guide
Revision	Rev A
Revision Date	December 2021

Automated Modular Workflow

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1 Workflow Overview

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1

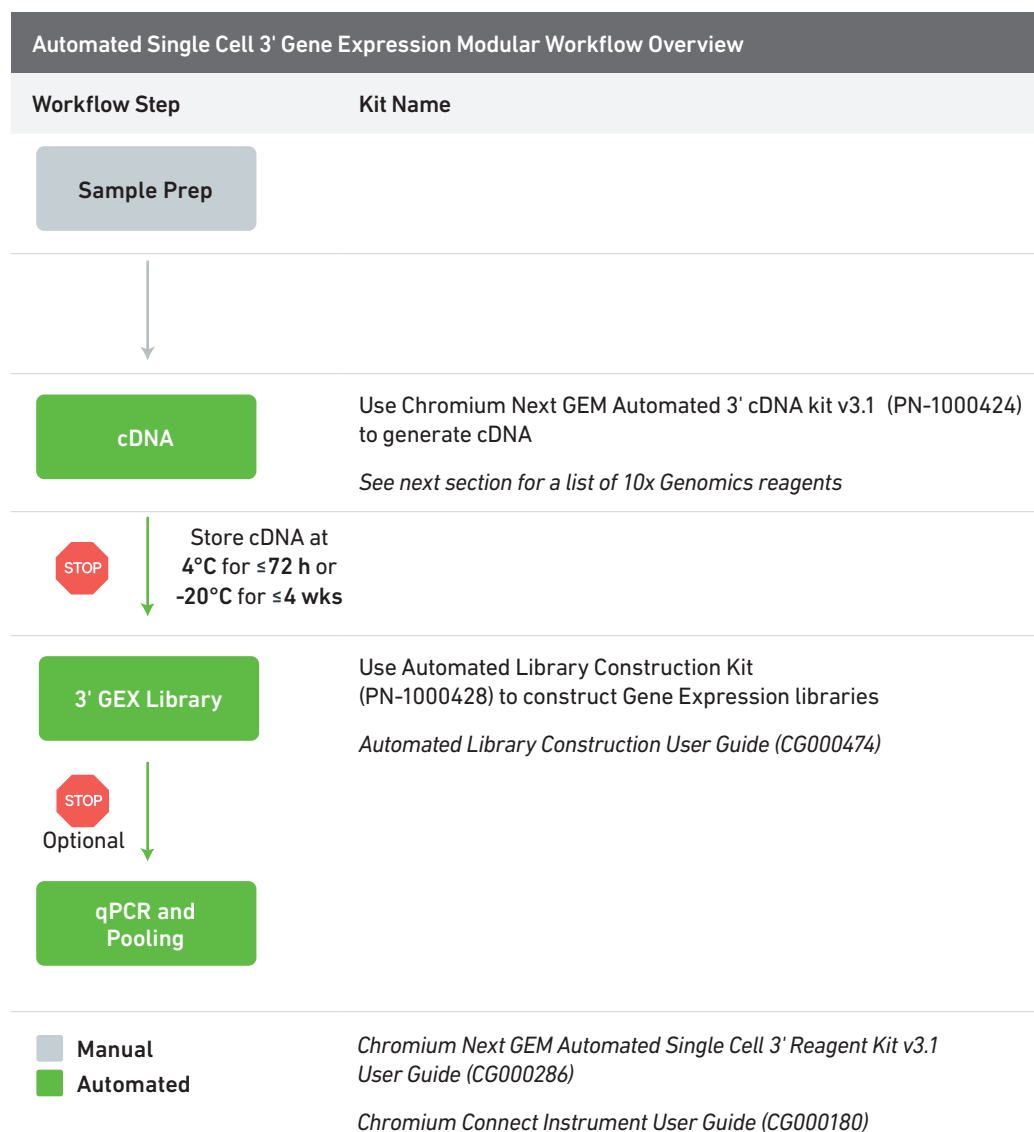
Protocol Steps & Timing

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1 Workflow Overview

This is a supplemental document that provides an overview of the Chromium Next GEM Automated Single Cell 3' 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 3' cDNA Kit v3.1 (PN-1000424). The cDNA can be stored at 4°C or -20°C or used directly for Single Cell 3' Library Construction using the Automated Library Construction Kit (PN-1000428) as described in the Automated Library Construction User Guide (CG000474).

For information on sample preparation, carrier loading and protocol guidelines, refer to the Chromium Next GEM Automated Single Cell 3' Reagent Kits v3.1 User Guide (CG000286) and the instrument user interface.

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



Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1

Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1, 24 rxns PN-1000424

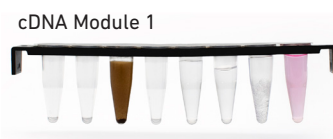
Reagent volumes and colors are different in each of the module types, not all module tubes contain reagents.

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

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

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

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

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

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

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Chromium Next GEM Automated Single Cell 3' Kit v3.1, cDNA Module 3, 24 rxns PN-1000397 (store at -20°C)

Chromium
Next GEM Automated
Single Cell 3' Kit v3.1, cDNA Module 3, 24 rxns

- | | | |
|--------------------------|---------------------------|----------------------|
| <input type="checkbox"/> | Single Cell cDNA Module 3 | 24 tube strips |
| | Template Switch Oligo | 6 tubes (PN-3000228) |

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Chromium Next GEM Automated Single Cell 3' cDNA Kit v3.1, 24 rxns PN-1000424

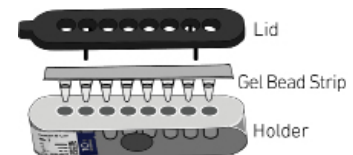
Chromium Next GEM Automated Single Cell 3' Gel Bead Kit v3.1, 24 rxns PN-1000137 (store at -80°C)

Chromium
Next GEM Automated
Single Cell 3' Gel Bead Kit v3.1, 24 rxns

Single Cell 3' v3.1 Gel Beads 3 tube strips

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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). Refer to Automated Library Construction User Guide (CG000474) 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	
Adaptor Ligation	~4 h Walk-away time
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	