10x GENOMICS

Supplemental User Guide | CG000791 | Rev B

Library Construction Kit C

24 Automated rxns / 32 Manual rxns

For use with:

Compatible Liquid Handlers

(refer to the 10x Genomics Support Website)

Notices

Document Number

CG000791 | Rev B

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

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Document Revision Summary

Document Number

CG000791

Title

Library Construction Kit C Supplemental User Guide

Revision

Rev A to Rev B

Revision Date

August, 2025

Description of Changes

- Updated to be inclusive of all compatible liquid handlers. Language included in multiple instances (pages 1, 5, 6, 7)
- Minor updates in language and format for consistency and clarity.

Table of Contents

Introduction

This is a supplemental document that provides information regarding the 10x Genomics Reagent Kits required for generating automated Single Cell 3' and 5' Gene Expression libraries from barcoded cDNA generated either using the manual or automated cDNA GEM-X workflow.

The library construction kits are designed to be automation friendly and provide sufficient reagent overages for 24 automated or 32 manual reactions per kit. An overview of the key steps is provided below.

cDNA Generation:

cDNA can be generated using either automation compatible 10x Reagent Kits or manually, using kits and methods specified in the compatible manual user guides:

- Chromium GEM-X Single Cell 3' Reagent Kits v4 User Guide (CG000731)
- Chromium GEM-X Single Cell 5' Reagent Kits v3 User Guide (CG000733)

Consult the 10x Genomics Support Website for the most current information regarding automation compatible cDNA kits, instruments, workflows, and recommended cDNA input.

The cDNA can be stored at 4°C for up to 72 hours, -20°C for ≤ 4 weeks, or used directly as input for automated library construction. Ensure that cDNA QC is performed as specified in the relevant 10x Genomics User Guides before using it for automated library construction.

Automated Library Construction:

Use cDNA as input for automated library construction using the indicated 10x Genomics Reagent Kits.

- Library Construction Kit C, Automated 24 rxns/Manual 32 rxns PN-1000774
- Dual Index Kit TT Set A, 96 rxns PN-1000215

This document provides information regarding the handling and preparation of the 10x Genomics reagents prior to using them for automated construction of GEM-X Single Cell 3' v4 and 5' v3 Gene Expression libraries. For guidance on automated library construction, follow compatible liquid handler manufacturer's user guide and user interface.

Compatible Liquid Handlers:

Consult the 10x Genomics Support website for the most current information.

Manual Library QC:

Once automated library construction is complete, perform library QC followed by sequencing, as specified in the relevant 10x Genomics User Guide. Consult the 10x Genomics Support website for data analysis and visualization guidance.

Protocol Steps & Timing: GEM-X 3' Gene Expression Library

The following protocol overview is based on the automated workflow on a compatible liquid handler. For the manual version of this kit, refer to CG000731 - Chromium GEM-X Single Cell 3' v4 Gene Expression User Guide.
For more details on protocol timing, consult automation platform-specific instrument user guide and/or contact the automation provider.

Steps	Timing	
Manual/Automated		
Cell Preparation (manual; dependent on Cell Type)	~1-1.5 h	
Step 1 – GEM Generation & Barcoding (manual or automated)	~1.5 h	STOP 4°C ≤72 h or -20°C ≤1 wk
Step 2 – Post GEM-RT Cleanup & cDNA Amplification (manual or automated)	~2.5 h	STOP 4°C ≤72 h or -20°C ≤4 wk
Use cDNA as input for automated library construction. cDNA may be generated using compatible automated or manual workflows.		
Reagent Preparation and Loading	~30 m	
Total Time *(dependent on use of on-deck thermal cycler)*	**~5.5 h**	
Automated		
Step 3 – 3' Gene Expression Library Construction		
Fragmentation, End Repair & A-tailing		
Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect		
Adaptor Ligation		
Post Ligation Cleanup- SPRIselect		
Sample Index PCR		
Post Sample Index PCR Double Sided Size Selection- SPRIselect		STOP 4°C ≤72 h or -20°C long-term
Post Library Construction QC (done off the instrument)		
Total Time *(dependent on number of samples)*	**~4.5-6.5 h**	

Protocol Steps & Timing: GEM-X 5' Gene Expression Library

The following protocol overview is based on the automated workflow on a compatible liquid handler. For the manual version of this kit, refer to CG000733 - Chromium GEM-X Single Cell 5' v3 Gene Expression User Guide.
For more details on protocol timing, consult automation platform-specific instrument user guide and/or contact the automation provider.

Steps	Timing	
Manual/Automated		
Cell Preparation (manual; dependent on Cell Type)	~1-1.5 h	
Step 1 – GEM Generation & Barcoding (manual or automated)	~1.5 h	STOP 4°C ≤72 h or -20°C ≤1 wk
Step 2 – Post GEM-RT Cleanup & cDNA Amplification (manual or automated)	~2.5 h	STOP 4°C ≤72 h or -20°C ≤4 wks
Use cDNA as input for automated library construction. cDNA may be generated using compatible automated or manual workflows.		
Reagent Preparation and Loading	~30 m	
Total Time *(dependent on using on-deck thermal cycler)*	**~5.5 h**	
Automated		
Step 3 – 5' Gene Expression Library Construction		
Fragmentation, End Repair & A-tailing		
Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect		
Adaptor Ligation		
Post Ligation Cleanup- SPRIselect		
Sample Index PCR		
Post Sample Index PCR Double Sided Size Selection- SPRIselect		STOP 4°C ≤72 h or -20°C long-term
Post Library Construction QC (done off the instrument)		
Total Time *(dependent on number of samples)*	**~4.5-6.5 h**	

Reagents Kits

Refer to SDS for handling and disposal information

Library Construction Kit C
Automated 24 rxns / Manual 32 rxns, PN-1000774
Shipped on dry ice
Store at -20°C

	#	PN
Fragmentation Enzyme	1	2001315
Fragmentation Buffer	1	2001316
Ligation Mix	1	2001317
DNA Ligase	1	2001318
Library Amp Mix	1	2001319

Dual Index Kit TT Set A
96 rxns, PN-1000215
Shipped on Dry Ice
Store at -20°C

	#	PN
Dual Index Kit Plate TT Set A	1	3000431

Reagent Preparation

Prepare reagents as specified below.

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	Fragmentation Buffer	2001316	Thaw, vortex, verify no precipitate, centrifuge.	–20°C
	Ligation Mix	2001317	Thaw, vortex, verify no precipitate, centrifuge.	–20°C
	Dual Index Plate TT Set A *Verify name & PN. Use indicated plate only*	3000431	—	–20°C
	Beckman Coulter SPRIselect Reagent	—	Manufacturer's recommendations.	—
Place on Ice	Fragmentation Enzyme *Ensure that Fragmentation Buffer and Fragmentation Enzyme from the same kit are used together. Lots are matched for optimal performance.*	2001315	Centrifuge briefly.	–20°C
	DNA Ligase	2001318	Centrifuge briefly.	–20°C
	Library Amp Mix	2001319	Vortex, centrifuge briefly.	–20°C
Obtain	Qiagen Buffer EB 9.54 ml for 24 rxns	—	Manufacturer's recommendations.	Ambient
	Prepare 80% Ethanol 53.5 ml for 24 rxns	—	Prepare fresh.	Ambient
	SPRI 7.04 ml for 24 rxns	—		

All aliquots of 10x Genomics reagents that are equilibrated to room temperature or placed on ice should be briefly centrifuged.

Proceed to Automated Library Construction

Use cDNA and the prepared 10x Genomics reagents for 3' and 5' Gene Expression library construction on a compatible liquid handler.

cDNA Input: A Single Cell Gene Expression library is generated using a fixed proportion (10 µl, 25%) of the total cDNA (40 µl) obtained using the manual workflow.

Note that irrespective of the total cDNA yield (ng), which may vary based on cell type, targeted cell recovery etc., this protocol has been optimized for a broad range of input mass (ng). The total number of SI PCR cycles should be optimized based the mass of cDNA present in a fixed volume (10 µl, 25%) of the total yield calculated during Post cDNA Amplification QC & Quantification. Consult the relevant upstream workflow user guide for more details.

- CG000731 - Chromium GEM-X Single Cell 3' v4 Gene Expression User Guide
- CG000733 - Chromium GEM-X Single Cell 5' v3 Gene Expression User Guide

Follow the compatible liquid handler user guide and user interface for instructions on automated library construction.

Recommended Thermal Cyclers

This method allows for on-deck thermal cycling. The following off-deck thermal cyclers have been validated by 10x Genomics for Chromium assays.

Description	Supplier	Part Number (US)
Biometra TAdvanced 96 SG/S	Analytik Jena*	846-x-070-241/846-x-070-251 *(x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
Mastercycler X50s/X50a	Eppendorf**	6311000010/6313000018
PTC Tempo Deepwell Thermal Cycler	Bio-Rad	12015392
C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module *(discontinued)*	Bio-Rad	1851197

For select instruments, ramp rate should be adjusted for all steps as described below:

**Analytik Jena Biometra TAdvanced 96 SG/S: 2°C/sec for both heating and cooling*

***Eppendorf Mastercycler X50s/X50a: 3°C/sec heating and 2°C/sec cooling*

TIPS

If using an off-deck thermal cycler, it should be pre-cooled to 4°C prior to use.