10x GENOMICS

Quick Reference Cards | CG000802 | Rev A

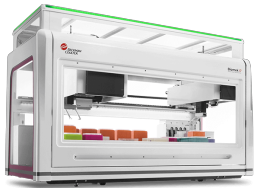
Chromium GEM-X Gene Expression Library Construction

on the Biomek i7 Hybrid Workstation

For use with:

10x Genomics, Library Construction Kit C, 24 Automated rxns/32 Manual rxns
Supplemental User Guide (CG000791)

Beckman Coulter Life Sciences, Biomek i7 Hybrid Workstation



BECKMAN COULTER Life Sciences

Document Revision Summary

Document Number

CG000802 | Rev A

Title

Chromium GEM-X Gene Expression Library Construction on the Beckman Biomek i7 Hybrid Workstation

Revision

Rev A

Revision Date

November 2024

Specific Changes

N/A

General Changes

N/A

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Overview

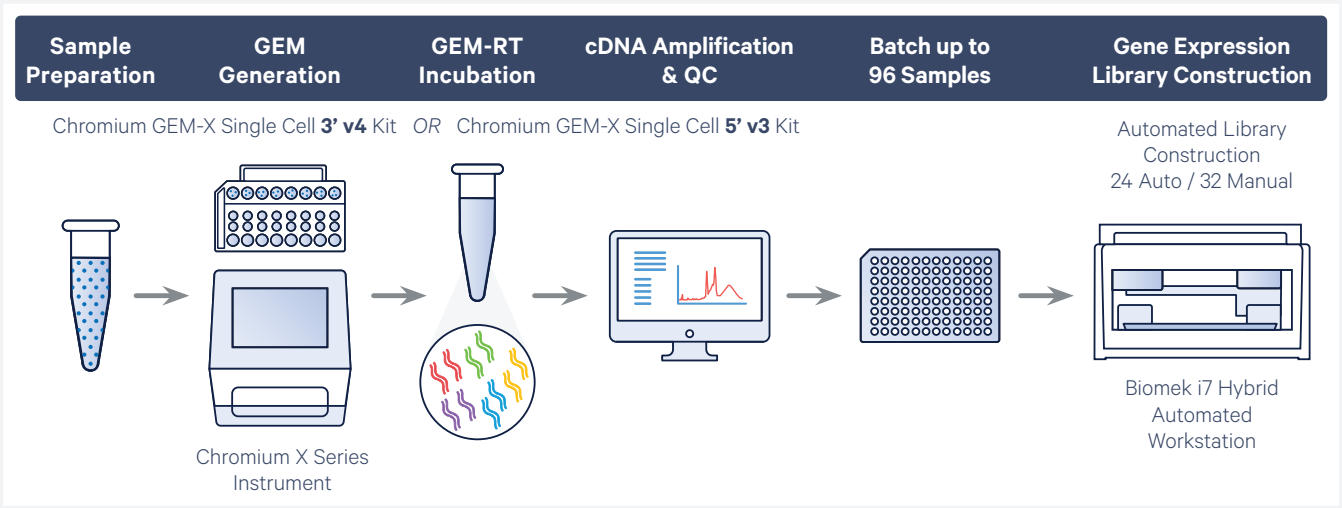

Figure 1. Start with a single cell suspension of cells. Run samples with a standard GEM-X singleplex workflow. After GEM generation using the Chromium X Series instrument from 10x Genomics, perform GEM-RT incubation, cDNA amplification and QC. Samples can be batched for automated gene expression library construction on the Biomek i7 Hybrid instrument from Beckman Coulter.

This quick reference card provides manual preparation instructions for users running the Chromium GEM-X Gene Expression Library Construction automated method on the Biomek i7 Hybrid Workstation.

Additional references for reagent and assay information from 10x Genomics can be found at https://www.10xgenomics.com/support/single-cell-gene-expression/documentation and include:

- Chromium GEM-X Single Cell 3' Reagent Kits v4 (10x Genomics, CG000731)
- Chromium GEM-X Single Cell 5' Reagent Kits v3 (10x Genomics, CG000733)
- Library Construction Kit C, 24 Auto/32 Manual User Guide (10x Genomics, CG000791)

Additional references for instrument operation and automated assay execution from Beckman Coulter Life Sciences can be found at https://www.beckman.com/support/technical-documents and include:

- GEM-X GEX Library Construction Method Setup Guide (2024-GBL-EN-106634)
- Automated Library Construction Test Procedure (Insert document information)

Consumables for Deck Setup

Labware, Reagents & Consumables

10x Genomics has created a kit specifically for automation use (Library Construction Kit C, Automated 24 rxns / Manual 32 rxns, PN-1000774) designed for 24 reactions. Additional required reagents and consumables are listed below:

Supplier	Description	Qty needed for 96 samples	Part Number
Reagents			
Thermo Fisher	Molecular Grade Nuclease-Free Water	30 ml	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	120 ml	E7023-500ML
Beckman Coulter	SPRIselect™ beads	23.7 ml	B23318
Qiagen	Qiagen Buffer EB	21.1 ml	19086
Consumables			
Beckman	BC190F, Empty Box	1	379498
	Tips, 190ul, Sterile fltrd (10 racks of 96)	18	B85911
	Tips, 1070ul, sterile fltrd (5 racks of 96)	1	B85955
	Quarter Reservoir (case of 48)	2	372790
Agilent	Reservoir, single cavity, polypropylene, 300 mL (25 pack)	1	201244-100
Bio-Rad	Hard-Shell® 96-well PCR Plates, low profile, thin wall, skirted (50 pack)	6	HSP9601
	Arched Auto-Sealing Lids for PCR Plates (pack of 4)	1	MSL2022
	Microseal® 'P' Reusable PCR Plate Sealing Pads (pack of 10)	1	MSP1001
Thermo Fisher	Nunc™ Microplate Lids (case of 60)	3	250003
	Abgene™ 96 Well 0.8mL polypropylene DeepWell™ (case of 50)	2	AB0765
	Nalgene™ Natural PPCO Micro Packaging Vials 2mL (case of 1000)	12	342800-0020
	Nalgene™ Natural PPCO Micro Packaging Vials 0.5mL (case of 1000)	0	342800-0005
Labware			
Beckman	Reservoir Frame	1	372795
	Tube Rack Holder 24-Position	1	373661
	Tube Rack Inserts, 11mm Diameter	1	373696
	Red heater/chiller 24-well block	1	Custom
Alpaqua	Magnum FLX magnet	1	A000400

Variable Reagent Volume Pipetting *(1 of 2)*

This information is also listed in the user interface. A "full" tube equates the use of a full unused kit reagent tube for 24 samples. When utilizing only a partial count from a new reagent tube (e.g., if 16 out of 24 reactions are used from the tube), always transfer the required volume to a new tube (size specified below). Ensure the original stock tube is returned to the -20°C freezer. See next page for an example on using this table.

			Red Reagent Block (10°C)			Black Tube Rack (Room Temp)	
# of Samples	Reactions / Tube	Tube Order	Frag Enzyme (µL) *0.5 ml Tube*	DNA Ligase (µL) *0.5 ml Tube*	Library Amp Mix (µL) *2 ml Tube*	Frag Buffer (µL) *0.5 ml Tube*	Ligation Mix (µL) *2 ml Tube*
8	8	First	114	124	455	67	419
16	16	First	219	228	855	128	768
24	Full (24)	First	303	318	1255	167	1130
32	Full	First	303	318	1255	167	1130
	8	Second	114	124	455	67	419
40	Full	First	303	318	1255	167	1130
	16	Second	214	228	855	123	768
48	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
56	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	8	Third	114	124	455	67	419
64	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	16	Third	214	228	855	123	768
72	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	Full	Third	303	318	1255	167	1130
80	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	Full	Third	303	318	1255	167	1130
	8	Fourth	114	124	455	67	419
88	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	Full	Third	303	318	1255	167	1130
	16	Fourth	214	228	855	123	768
96	Full	First	303	318	1255	167	1130
	Full	Second	303	318	1255	167	1130
	Full	Third	303	318	1255	167	1130
	Full	Fourth	303	318	1255	167	1130

Variable Reagent Volume Pipetting *(2 of 2)*

Cleanup Reservoirs (Room Temp)			
# of Samples	SPRIselect™ beads (mL) *QTR Reservoir*	Buffer EB (mL) *QTR Reservoir*	EtOH (mL) *Full Reservoir*
8	3.3	4.5	37.8
16	5.2	6.9	45.7
24	7.0	9.5	53.5
32	8.9	10.8	61.3
40	10.7	12.1	69.2
48	12.6	13.4	77.0
56	14.4	14.7	84.8
64	16.3	15.9	92.7
72	18.1	17.2	100.5
80	20.0	18.5	108.3
88	21.8	19.8	116.2
96	23.7	21.1	124.0

Example with 40 samples

Red Reagent Block: *This requires 1 full tube with minimum volumes listed that equate to 24 reactions. Additionally, one partial tube is required per reagent listed, equating to 16 reactions.*

Frag Enzyme:	• 1 full 0.5 mL tube (303 µL) • 1 partial 0.5 mL tubes (219 µL)
DNA Ligase:	• 1 full 0.5 mL tube (318 µL) • 1 partial 0.5 mL tubes (229 µL)
Library Amp Mix:	• 1 full 2 mL tube (1255 µL) • 1 partial 2 mL tubes (855 µL)

Black Tube Rack: *This requires 1 full tube with minimum volumes listed that equate to 24 reactions. Additionally, one partial tube is required per reagent listed, equating to 16 reactions.*

Frag Buffer:	• 1 full 0.5 mL tube (167 µL) • 1 partial 0.5 mL tube (128 µL)
Ligation Mix:	• 1 full 2 mL tube (1130 µL) • 1 partial 2 mL tube (768 µL)

Cleanup reservoir volumes: *volumes listed for 40 samples are the minimum volumes required for the automated assay.*

SPRIselect™ beads:	• Quarter reservoir (10.7 mL)
Buffer EB:	• Quarter reservoir (12.1 mL)
EtOH:	• Full reservoir (69.2 mL)

Off-Deck Thermal Cycler Protocols

Fragmentation, End Repair & A-Tailing

Pre-chill thermal cycler prior to starting

Lid Temperature	Rxn Volume	Run Time
65°C	50 µl	~35 min
Step	**Temperature**	**Time hh:mm:ss**
Pre-cool block	4°C	Hold
Fragmentation	32°C	00:05:00
End Repair & A-Tailing	65°C	00:30:00
Hold	4°C	Hold

Adaptor Ligation

Lid Temperature	Rxn Volume	Run Time
30°C	100 µl	15 min
Step	**Temperature**	**Time hh:mm:ss**
1	20°C	00:15:00
2	4°C	Hold

Sample Index PCR

Lid Temperature	Rxn Volume	Run Time
105°C	100 µl	~30 min
Step	**Temperature**	**Time hh:mm:ss**
1	98°C	00:00:45
2	98°C	00:00:20
3	54°C	00:00:30
4	72°C	00:00:20
5	Go to Step 2, see below for # of cycles	
6	72°C	00:01:00
7	4°C	Hold

Recommended Cycle Numbers

cDNA Input	Total Cycles
0.25-50 ng	14-16
50-250 ng	12-14
250-600 ng	10-12
600-1,100 ng	8-10
1,100-1,500 ng	6-8
>1,500 ng	5

Technical Support

For automation or method-related issues, contact Beckman Coulter Life Sciences North America:

LSHotline@Beckman.com

+1 800 369-0333

Within North America:

- Press 3 for Technical Support
  then # (to skip entering a system ID)
  then 6 (to select the Genomics product line)
- 8:00am – 8:00pm ET on weekdays

Within EU:

- LSEuropeOrders@beckman.com

For region-specific general email and phone enquiry contact information, visit https://www.beckman.com/contact-us and select the appropriate location

For reagent issues, contact 10x Genomics:

support@10xgenomics.com

+1 925 401-7300

Within North America:

- Press 2 for Technical Support
- 9:00 am – 8:00 pm ET on weekdays

Customers who perform the automated assay without modification to the method, hardware, or unexpired reagents will have full support by 10x Genomics.

Customers who modify the method, hardware, or reagent to customize their 10x automated assay will receive limited support by 10xGenomics.

Provide Technical Support with the Following Information

- Customer account and contact name
- Product Name and/or PN
- Reagent Kit Lot #
- System ID or SN
- Problem statement with Error Codes (if applicable)
- Exported instrument logs
  - Launch Automation Troubleshooter by going to Windows Start button > Beckman Coulter > Automation Troubleshooter.
  - Click the “Export System Info” button.