User Guide | CG001689 | Rev C

GEM-X Epi Multiome for ATAC + Gene Expression

For use with:

GEM-X Epi Multiome
16 samples PN-1000979 | 4 samples PN-1000980

GEM-X Epi Multiome Chip Kit, 4 chips, PN-1000951

Single Index Kit N Set A, 96 rxns, PN-1000212

Dual Index Kit TT Set A, 96 rxns, PN-1000215

Nuclei Isolation Kit with RNase Inhibitor v2, 16 rxns, PN-1001101

RNase Inhibitor 40x, PN-1000887 is also available for purchasing separately

Take 1 minute to evaluate this protocol. Scan this code or click here.

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Notices

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Support

Email: support@10xgenomics.com
10x Genomics, Inc.
6230 Stoneridge Mall Road
Pleasanton, CA

Document Revision Summary

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CG001689

Title

GEM-X Epi Multiome User Guide

Revision

Rev C

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May 11, 2026

Description of Changes

Corrected Ligation Mix volume in 4X and 8X columns in Adaptor Ligation on page 88

Updated for general minor consistency of language and terms throughout.

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GEM-X Epi Multiome Reagents

10x reagents are listed in the same order as their placement in the kits.

Refer to SDS for handling and disposal information.

GEM-X Epi Multiome, 16 samples PN-1000979

GEM-X Epi Multiome GEM Kit
16 rxns, PN-1000945
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	Barcoding Reagent C	2001558		Barcoding Enzyme B	2001562
	Template Switch Oligo B	2001027		Reducing Agent B	2000087
	Cleanup Buffer	2000088		Cleanup Buffer	2000088
	Quenching Agent	2001679	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X Epi Multiome AMP Kit
16 rxns, PN-1000947
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	Pre-Amp Mix	2000270		Pre-Amp Primers	2000271
	Amp Mix	2000047		Amp Mix	2000047
	cDNA Primers	2000089		SI-PCR Primer B	2000128
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X
Multiome Gel Bead Kit
16 rxns, PN-1000943
Shipped on dry ice
Store at -80°C

	#	PN
Single Cell Multiome Gel Bead	2	2001514

Dynabeads™ MyOne™ SILANE
PN-2000048
Shipped at ambient temperature
Store at 4°C

	#	PN
Dynabeads MyOne SILANE	1	2000048

GEM-X Epi Multiome ATAC Kit, 16 rxns PN-1000949

GEM-X Epi Multiome ATAC Kit
16 rxns, PN-1000949
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
Blue	20X Nuclei Buffer	2000207	Blue	20X Nuclei Buffer	2000207
Green	ATAC Buffer C	2001581	Green	ATAC Enzyme B	2001582
Blue	RNase Inhibitor 40x	2001488	-	*empty*	-
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

Library Construction Kit C, 16 rxns PN-1000694*

Library Construction Kit C
16 rxns, PN-1000694
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
Purple	Fragmentation Enzyme	2000090	Purple	Fragmentation Buffer	2000091
Orange	Ligation Mix	2001109	Orange	DNA Ligase	220110
Clear	Library Amp Mix	2000531	-	*empty*	-
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

*Alternatively, use Library Construction Kit C, Automated 24 rxns/ Manual 32 rxns PN-1000774

This document describes the use of this kit for manual gene expression library construction. For details on using this kit for automated library construction, refer to Supplemental User Guide Library Construction Kit C (CG000791).

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

Cap Color	Reagent	PN	Cap Color	Reagent	PN
Purple	Fragmentation Enzyme	2001315	Purple	Fragmentation Buffer	2001316
Orange	Ligation Mix	2001317	Orange	DNA Ligase	2001318
Clear	Library Amp Mix	2001319	-	*empty*	-
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X Epi Multiome, 4 samples PN-1000980

GEM-X Epi Multiome GEM Kit
4 rxns, PN-1000946
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	Barcoding Reagent C	2001558		Barcoding Enzyme B	2001562
	Template Switch Oligo B	2001027		Reducing Agent B	2000087
	Cleanup Buffer	2000088	-	*empty*	-
	Quenching Agent	2001679	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X Epi Multiome AMP Kit
4 rxns, PN-1000948
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	Pre-Amp Mix	2000270		Pre-Amp Primers	2000271
	Amp Mix	2000047	-	*empty*	-
	cDNA Primers	2000089		*SI-PCR Primer B*	2000128
-	empty	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X
Multiome Gel Bead Kit
4 rxns, PN-1000944
Shipped on dry ice
Store at -80°C

	#	PN
GEM-X Multiome Gel Beads	0.5	2001514

Dynabeads™ MyOne™ SILANE
PN-2000048
Shipped at ambient temperature
Store at 4°C

	#	PN
Dynabeads MyOne SILANE	1	2000048

GEM-X Epi Multiome ATAC Kit, 4 rxns PN-1000950

GEM-X Epi Multiome ATAC Kit
4 rxns, PN-1000950
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	20X Nuclei Buffer	2000207		20X Nuclei Buffer	2000207
	ATAC Buffer C	2001581		ATAC Enzyme B	2001583
	RNase Inhibitor 40x	2001488	-	*empty*	-
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

Library Construction Kit C, 4 rxns PN-1000689

Library Construction Kit C
4 rxns, PN-1000689
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
	Fragmentation Enzyme	2000104		Fragmentation Buffer	2000091
	Ligation Mix	2001109		DNA Ligase	220131
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-
-	*empty*	-	-	*empty*	-

GEM-X Epi Multiome Chip Kit, 4 chips PN-1000951

Chromium GEM-X
Partitioning Oil B
Shipped at ambient temperature
Store at ambient temperature

Cap	Reagent	#	PN
● (gray)	Partitioning Oil B	6	2001213

Chromium GEM-X
Recovery Agent
Shipped at ambient temperature
Store at ambient temperature

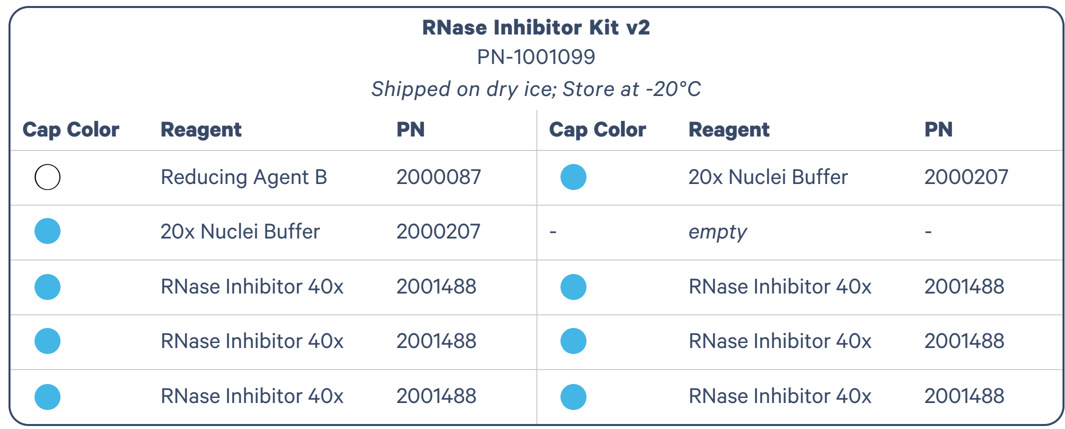
Cap	Reagent	#	PN
○	Recovery Agent	6	220016

GEM-X Epi Multiome
Chip & Gaskets
Shipped at ambient temperature
Store at ambient temperature

	#	PN
GEM-X MO Chip	4	2001485
X/iX Chip Gasket, 2-pack	2	3000656

Nuclei Isolation Kit with RNase Inhibitor v2, PN-1001101

RNase Inhibitor Kit v2, PN-1001099



RNase Inhibitor Kit v2
PN-1001099
Shipped on dry ice; Store at -20°C

Cap Color	Reagent	PN	Cap Color	Reagent	PN
○	Reducing Agent B	2000087	● (blue)	20x Nuclei Buffer	2000207
● (blue)	20x Nuclei Buffer	2000207	-	*empty*	-
● (blue)	RNase Inhibitor 40x	2001488	● (blue)	RNase Inhibitor 40x	2001488
● (blue)	RNase Inhibitor 40x	2001488	● (blue)	RNase Inhibitor 40x	2001488
● (blue)	RNase Inhibitor 40x	2001488	● (blue)	RNase Inhibitor 40x	2001488

RNase Inhibitor 40x, Kit PN-1000887 (tube PN-2001488) can also be purchased separately

Single Index Kit N Set A, 96 rxns PN-1000212

Single Index Kit N Set A 96 rxns, PN-1000212 *Shipped on dry ice* *Store at −20°C*	#	PN
Single Index Plate N Set A	1	3000427

Dual Index Kit TT Set A, 96 rxns PN-1000215

Dual Index Kit TT Set A 96 rxns, PN-1000215 *Shipped on dry ice* *Store at −20°C*	#	PN
Dual Index Plate TT Set A	1	3000431

Reagent Kit Summary

Orderable Kit	PN	Kits	PN	Quantity
GEM-X Epi Multiome, 16 samples	1000979	GEM-X Epi Multiome Gel Bead Kit, 16 rxns	1000943	1
		GEM-X Epi Multiome GEM Kit, 16 rxns	1000945	1
		GEM-X Epi Multiome Amp Kit, 16 rxns	1000947	1
		Dynabeads™ MyOne™ SILANE	2000048	1
		GEM-X Epi Multiome ATAC Kit, 16 rxns	1000949	1
		Library Construction Kit C, 16 rxns	1000694 *(orderable separately)*	1
GEM-X Epi Multiome, 4 samples	1000980	GEM-X Epi Multiome Gel Bead Kit, 4 rxns	1000944	1
		GEM-X Epi Multiome GEM Kit, 4 rxns	1000946	1
		GEM-X Epi Multiome Amp Kit, 4 rxns	1000948	1
		Dynabeads™ MyOne™ SILANE	2000048	1
		GEM-X Epi Multiome ATAC Kit, 4 rxns	1000950	1
		Library Construction Kit C, 4 rxns	1000689	
GEM-X Epi Multiome Chip Kit, 4 chips	1000951	GEM-X Epi Multiome Chip Kit, 4 chips	1000951	1
Nuclei Isolation Kit with RNase Inhibitor v2	1001101	Chromium Nuclei Isolation Consumables Kit, 16 rxns	1000448	1
		Chromium Nuclei Isolation Reagents Kit, 16 rxns	1000447	1
		RNase Inhibitor v2	1001099	1
RNase Inhibitor 40x, Kit PN-1000887 (tube PN-2001488) can also be purchased separately				
Single Index Kit N Set A, 96 rxns	1000212	Single Index Kit N Set A, 96 rxns	1000212	1
Dual Index Kit TT Set A, 96 rxns	1000215	Dual Index Kit TT Set A, 96 rxns	1000215	1

10x Genomics Accessories

Product	PN (Orderable)	PN (Item)
10x Vortex Adapter	120251	330002
10x Magnetic Separator B*	1000709 *(Chromium X/iX Accessory Kit)/* 1000821 *(Chromium X Series Accessory Kit)/* 1000707 *(GEM-X Transition Kit)*	2001212
Chromium X Series Chip Holder (also referred to as Chromium X Series Chip Holder)		3000598

**10x Magnetic Separator (PN-230003) and 10x Magnetic Separator B (PN-2001212) can be used interchangeably.*

Third-Party Items

Successful execution of this workflow requires third-party reagents, kits, and equipment in addition to those provided by 10x Genomics. All third-party reagents and consumables should be obtained prior to starting this library construction workflow.

Refer to the **Protocol Planner CG001688** for a detailed list of the following third-party items:

- Additional reagents, kits, and equipment
- Recommended pipette tips
- Recommended thermal cyclers

10x Genomics has tested all items listed in the Protocol Planner. These items perform optimally with the assay. Substituting materials may adversely affect assay performance.

Protocol Steps & Timing

Steps	Timing	Stop & Store	
Nuclei Isolation			
Dependent on Cell Type	~1-2 h		
Choose either Individual (Step 1) or Scalable (Step 2) Transposition			
Step 1: Individual Transposition (page 38)			
1.1 Prepare Transposition Mix (page 43)	10 min		
1.2 Isothermal Incubation (page 43)	30-60 min		
1.3 Post Transposition Wash (page 44)	10 min		
OR			
Step 2: Scalable Transposition (page 45)			
2.1 Prepare Transposition Mix (page 50)	10 min		
2.2 Isothermal Incubation (page 50)	30-60 min		
2.3 Post Transposition Wash (page 51)	10 min		
Step 3: GEM Generation & Barcoding (page 52)			
3.1 Prepare Master Mix (page 54)	10 min		
3.2 Load GEM-X Chip (page 58)	10 min		
3.3 Run the Chromium X Series Instrument (X/iX) (page 61)	6 min		
3.4 Transfer GEMs (page 61)	3 min		
3.5 GEM Incubation (page 63)	75 min		
3.6 Quenching Reaction (page 63)	5 min	STOP	−80°C ≤ 4 week
Step 4: Post GEM Incubation Cleanup (page 64)			
4.1 Post GEM Incubation Cleanup – Dynabeads (page 66)	35 min		
4.2 Post GEM Incubation Cleanup – SPRIselect (page 68)	15 min		
Step 5: Pre Amplification PCR (page 69)			
5.1 Prepare Pre Amplification Mix (page 70)	10 min		
5.2 Pre Amplification PCR (page 71)	30 min	STOP	4°C ≤18 h
5.3 Pre Amplification SPRI Cleanup (page 71)	15 min	STOP	4°C ≤72 h or −20°C long-term
Step 6: ATAC Library Construction (page 73)			
6.1 Sample Index PCR (page 74)	45 min		
6.2 Post Sample Index Double Sided Size Selection – SPRIselect (page 75)	20 min	STOP	4°C ≤72 h or −20°C long-term
6.3 Post Library Construction QC (page 77)	60 min		
Step 7: cDNA Amplification (page 78)			
7.1 cDNA Amplification (page 79)	40 min	STOP	4°C ≤72 h or −20°C ≤1 week

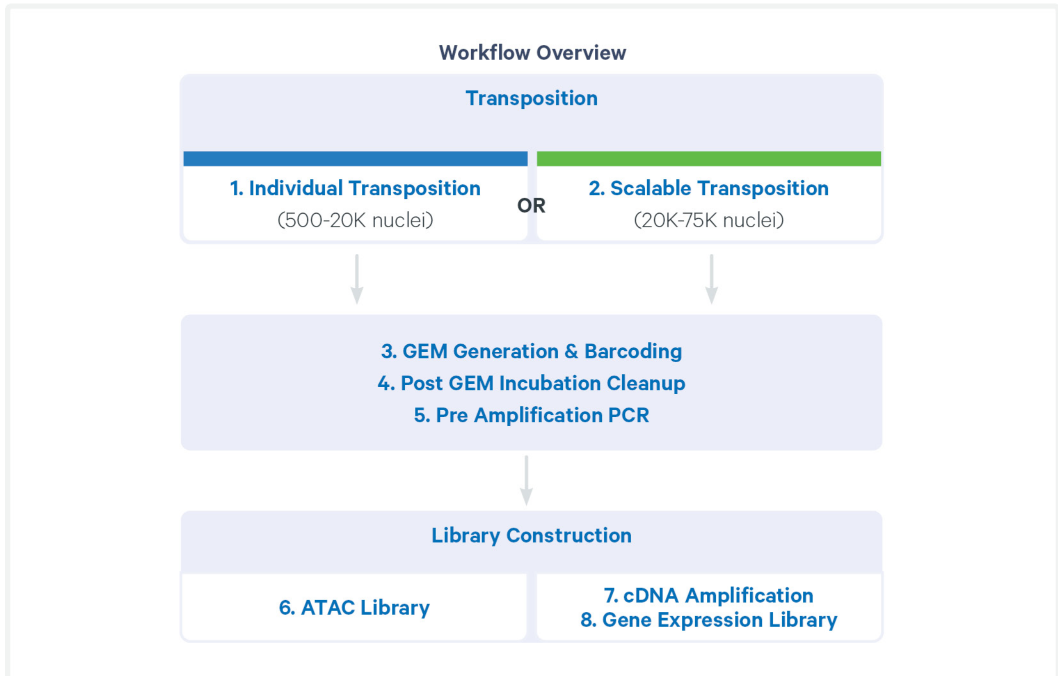
Steps	Timing	Stop & Store	
7.2 cDNA Cleanup – SPRIselect (page 80)	15 min	STOP	4°C ≤72 h or −20°C ≤4 weeks
7.3 cDNA QC & Quantification (page 81)	50 min		
Step 8: Gene Expression Library Construction (page 83)			
8.1 Fragmentation, End Repair & A-tailing (page 86)	45 min		
8.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect (page 87)	30 min		
8.3 Adaptor Ligation (page 88)	25 min		
8.4 Post Ligation Cleanup – SPRIselect (page 89)	20 min		
8.5 Sample Index PCR (page 90)	40 min	STOP	4°C ≤72 h
8.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect (page 91)	30 min	STOP	4°C ≤72 h or -20°C long term
8.7 Post Library Construction QC (page 92)	50 min		

Stepwise Objectives

GEM-X Epi Multiome provides a comprehensive, scalable multiomic approach for simultaneously profiling epigenomic landscape and gene expression in the same single nuclei. This is achieved by transposing nuclei in a bulk solution. Using a microfluidic chip, the nuclei are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs). A pool of ~750,000 10x Barcodes is sampled to separately and uniquely index the transposed DNA and cDNA of each individual nucleus. ATAC and gene expression (GEX) libraries are generated from the same pool of pre-amplified transposed DNA/cDNA and sequenced. The 10x Barcodes in each library type are used to associate individual reads back to the individual partitions, and thereby, to each individual nucleus.

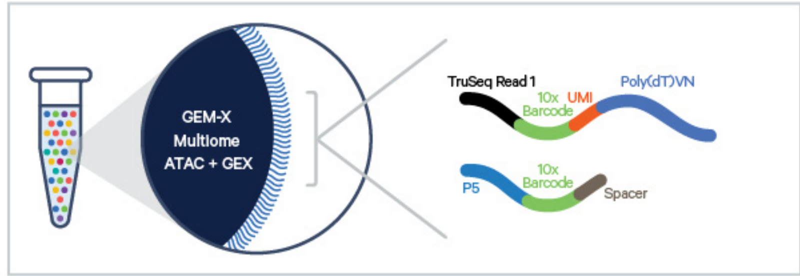
Step 1 or Step 2: Transposition

Nuclei suspensions are incubated in a Transposition Mix that includes a Transposase. The Transposase enters the nuclei and preferentially fragments the DNA in open regions of the chromatin. Simultaneously, adapter sequences are added to the ends of the DNA fragments. Depending on starting nuclei input and experimental goals, choose either Individual or Scalable Transposition as per the Transposition Selection Guidelines on page 36.

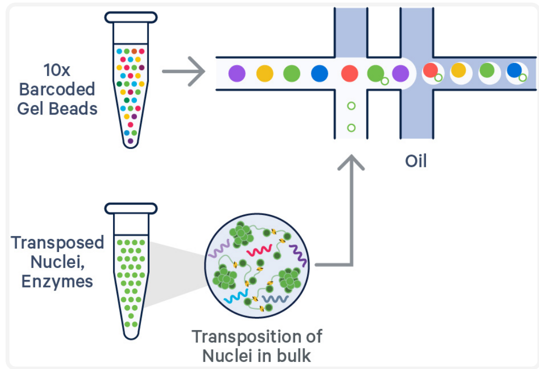


Step 3: GEM Generation & Barcoding

GEM-X Epi Multiome Gel Beads include a poly(dT) sequence that enables production of barcoded, full-length cDNA from poly-adenylated mRNA for GEX library and a Spacer sequence that enables barcode attachment to transposed DNA fragments for ATAC library.



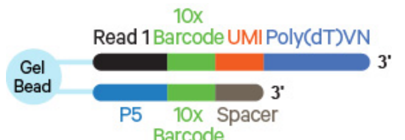
GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a GEM-X MO Chip. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder largely contain a single nucleus.

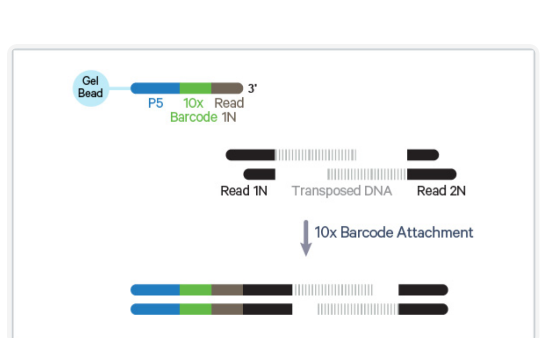


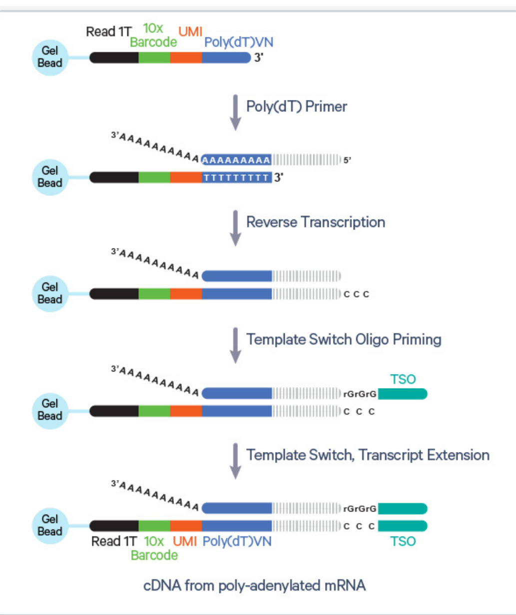
Upon GEM generation, the Gel Bead is dissolved. Oligonucleotides containing an Illumina® P5 sequence, a 16 nt 10x Barcode (for ATAC), and a Spacer sequence are released. In the same partition, primers containing an Illumina® TruSeq Read 1 (read 1 sequencing primer), 16 nt 10x Barcode (for GEX), 12 nt unique molecular identifier (UMI), and a 30 nt poly(dT) sequence are also released. The primers are mixed with the nuclei lysate containing transposed DNA fragments, mRNA, and Master Mix, that includes reverse transcription (RT) reagents.

Incubation of the GEMs produces 10x Barcoded DNA from the transposed DNA (for ATAC) and 10x Barcoded, full-length cDNA from poly-adenylated mRNA (for GEX). This is followed by a quenching step that stops the reaction.

Inside Individual GEMs







Step 4: Post GEM Cleanup

GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the cell barcoded products from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers.

Step 5: Pre Amplification PCR

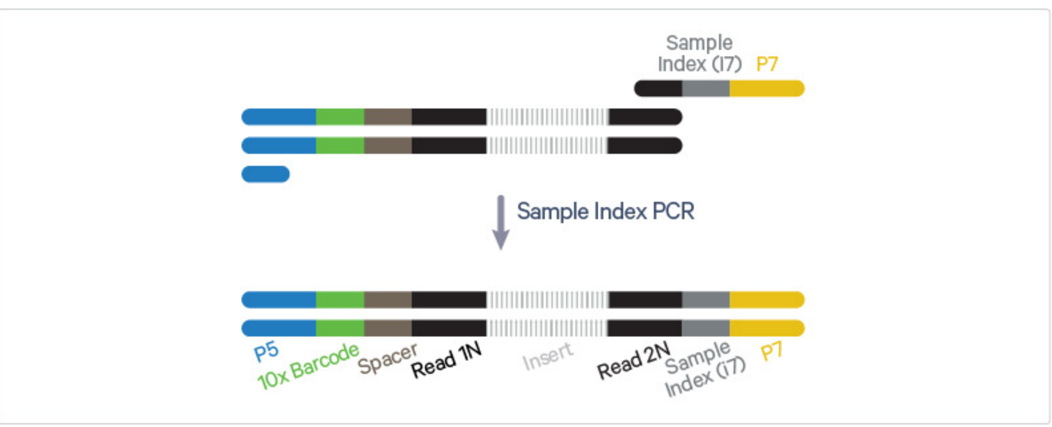
Barcoded transposed DNA and barcoded full length cDNA from poly-adenylated mRNA are amplified via PCR to fill gaps and for generating sufficient mass for library construction. The pre-amplified product is used as input for both ATAC library construction and cDNA amplification for gene expression library construction.

Pooled pre-amplification PCR

Step 6: ATAC Library Construction

P7 and a sample index are added to pre-amplified transposed DNA during ATAC library construction via PCR. The final ATAC libraries contain the P5 and P7 sequences used in Illumina® amplification.

ATAC Library Construction



Step 7: cDNA Amplification

Barcoded, full-length pre-amplified cDNA is amplified via PCR to generate sufficient mass for gene expression library construction.

Step 8: Gene Expression Library Construction

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. P5, P7, i7 and i5 sample indexes, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final gene expression libraries contain the P5 and P7 primers used in Illumina® amplification.

cDNA Amplification & Gene Expression Library Construction

Step 9: Sequencing

Chromium Single Cell Multiome ATAC libraries comprise double stranded DNA with standard Illumina® paired-end constructs which begin with P5 and end with P7.

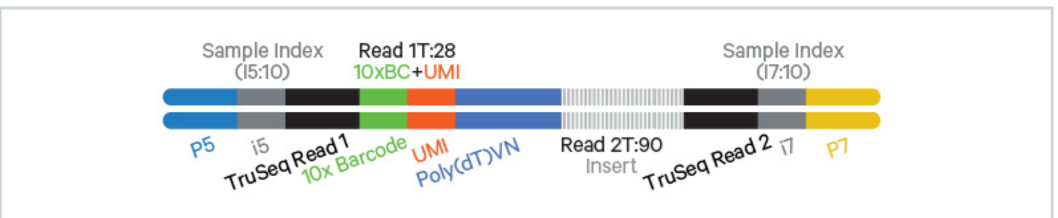
Sequencing these libraries produces a standard Illumina® BCL data output folder that includes paired-end Read 1N and Read 2N used for sequencing the DNA insert, along with the 8 bp sample index in the i7 read and 16 bp 10x Barcode sequence in the i5 read.

Chromium Single Cell Multiome ATAC Library

Chromium Single Cell Multiome Gene Expression libraries comprise cDNA insert with standard Illumina® paired-end constructs which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder.

TruSeq Read 1 is used to sequence 16 bp 10x Barcodes and 12 bp UMI, while 10 bp i5 and i7 sample index sequences are the sample index reads. TruSeq Read 2 is used to sequence the insert.

Chromium Single Cell Multiome Gene Expression Library



Tips & Best Practices

Icons

Tips & Best Practices section for additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section for additional guidance

GEM-X specific protocol updates

Emulsion-safe Plastics

- Use 10x Genomics validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

Nuclei Concentration

- See the nuclei input concentration for Individual Transposition on page 38. The entire volume of nuclei after Individual Transposition is used for GEM Generation.
- See the nuclei input concentration for Scalable Transposition on page 45.

  Nuclei concentration is determined after Scalable Transposition. The transposed nuclei input concentration is specified in GEM Generation & Barcoding on page 52.
- The optimal input nuclei concentration depends upon the desired nuclei recovery target. For details, see the Transposition and GEM Generation steps.

Optimal Input Concentration	Nuclei Recovery Target
700-1,200 nuclei/ µl	500-20,000 nuclei
1,300-1,600 nuclei/ µl	10,000-20,000 nuclei

- The presence of debris in the suspension may reduce the recovery rate. Consult the following documents for more information on preparing cells:
  - 10x Genomics Single Cell Protocols Cell Preparation Handbook (Documents CG00053)
  - Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failure (Document CG000479)

Multiplet Rate (%)	# of Nuclei Loaded	# of Nuclei Recovered
~0.20%	~725	~500
~0.40%	~1,450	~1,000
~0.80%	~2,900	~2,000
~1.20%	~4,350	~3,000
~1.60%	~5,800	~4,000
~2.00%	~7,250	~5,000
~2.40%	~8,700	~6,000
~2.80%	~10,150	~7,000
~3.20%	~11,600	~8,000
~3.60%	~13,050	~9,000
~4.00%	~14,500	~10,000
~4.40%	~15,950	~11,000
~4.80%	~17,400	~12,000
~5.20%	~18,850	~13,000
~5.60%	~20,300	~14,000
~6.00%	~21,750	~15,000
~6.40%	~23,200	~16,000
~6.80%	~24,650	~17,000
~7.20%	~26,100	~18,000
~7.60%	~27,550	~19,000
~8.00%	~29,000	~20,000

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage.
- Calculate reagent volumes with 10% excess of reaction volumes.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

50% Glycerol Solution

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.

  OR

- Prepare 50% glycerol solution:

a. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.

b. Filter through a 0.2 µm filter.

c. Store at −20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

GEM-X Chromium X Series Chip Holder

- Chromium X/iX or X Series Chip Holders encase GEM-X Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the slider on the back (right side) of the holder toward the middle to unlock the lid and return the holder to a flat position.

GEM-X Chip Handling

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in **≤24 h**.
- Execute steps without pause or delay, unless indicated. When using multiple chips, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the top NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to wetting failures.
- Minimize the distance that a loaded chip is moved to reach the compatible Chromium X series instrument (X/iX).
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion. DO NOT pick up the assembled chip holder by the holder lid as this could accidentally lead to spillage or dropping of the chip holder.

GEM-X Chip & Holder Assembly with Gasket

- Close the holder lid. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket.
- Open the chip holder.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the chip is inserted under the guide on the holder.
- Depress the right hand side of the chip until the spring-loaded clip engages.
- Keep the assembled unit with the attached gasket until ready for dispensing reagents.

  One of the rows of the gasket will not align with the GEM-X chip wells. This is expected and will not impact the assay.

  A generic image representative of the GEM-X Chip is shown below. The Chromium X/iX Chip Holder is also referred to as Chromium X Series Chip holder.

GEM-X Chip Loading

- Place the assembled chip and holder flat (gasket attached) on the bench with the lid open.
- Dispense at the bottom of the wells without introducing bubbles.

- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load GEM-X Chip on page 58 for specific instructions.

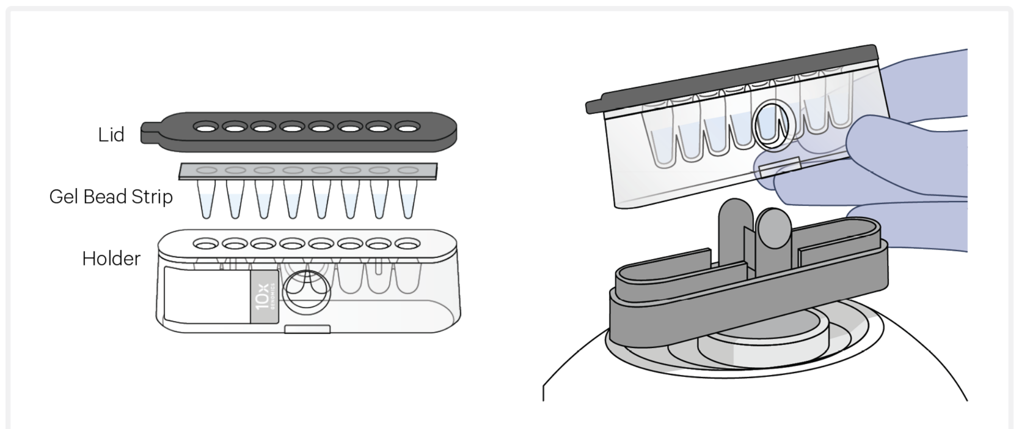
GEM-X Chromium X Series Instrument Firmware

- Firmware version 2.0.0 or higher is required in the Chromium Series instrument (X/iX) to run the GEM-X chips.
- The current firmware version of the instrument will be displayed on the instrument homescreen.
- Consult the Chromium X Series Instrument User Guide with Readiness Test (CG000396) for detailed instructions on updating the firmware.
- After an instrument run, to troubleshoot any potential clogs and failures, it is recommended to photograph the GEMs in the chip recovery wells and the pipette tips during GEM retrieval (or the tubes after GEM transfer).

Gel Bead Handling

- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- After removing the Gel Bead strip from the packaging, equilibrate the Gel Bead strip to **room temperature** for at least **30 min** before use.
- Store unused Gel Beads at **−80°C** and avoid more than 12 freeze-thaw cycles.

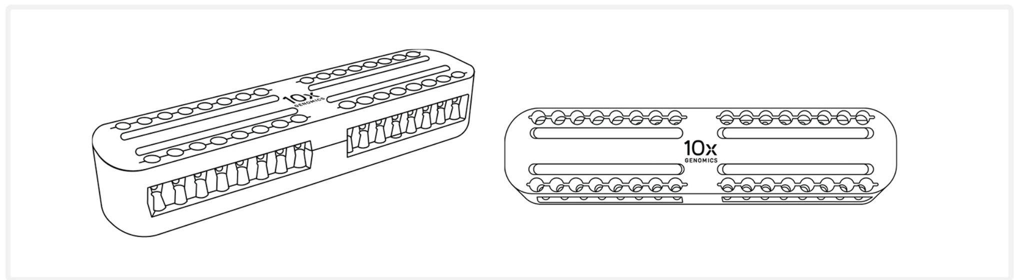
  DO NOT store Gel Beads at **−20°C**.
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for **~5 sec** after removing from the holder. Confirm there are no bubbles at the bottom of tubes and the liquid levels look even. Place Gel Bead strip back in the holder and secure the holder lid.
- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly. DO NOT reuse pipette tips and do not combine aliquots to avoid cross contamination.



10x Magnetic Separator

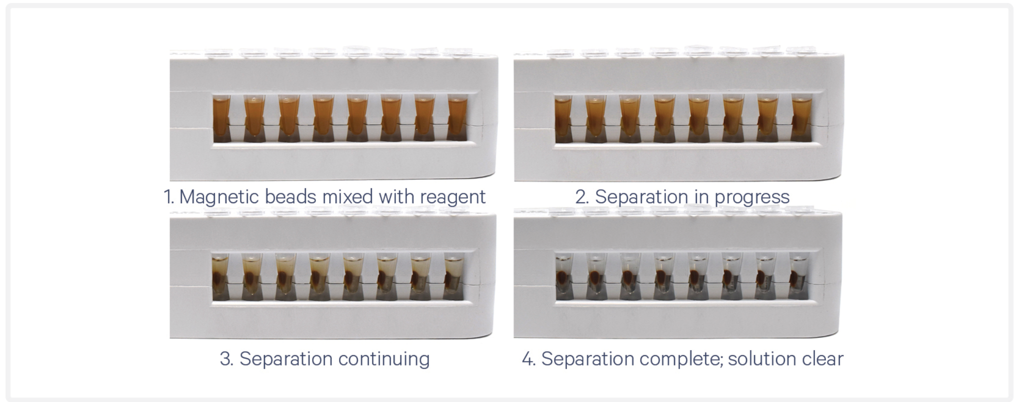
- Representative illustrations are provided below - actual appearance of magnetic separator may vary. Guidance applies to all 10x Magnetic Separators.

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•High) or low (magnet•Low) positions.
- If using MicroAmp 8-Tube Strips, use the high position (magnet•High) only throughout the protocol.



Magnetic Bead Cleanup Steps

- During magnetic bead based cleanup steps that specify waiting "until the solution clears", visually confirm clearing of solution before proceeding to the next step. See image below for an example.
- The time needed for the solution to clear may vary based on specific step, reagents, volume of reagents etc.
- Images below are representative - actual color of magnetic separator may vary. Guidance applies to all 10x Magnetic Separators.



1. Magnetic beads mixed with reagent
2. Separation in progress
3. Separation continuing
4. Separation complete; solution clear

SPRIselect Cleanup & Size Selection

- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.

- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.

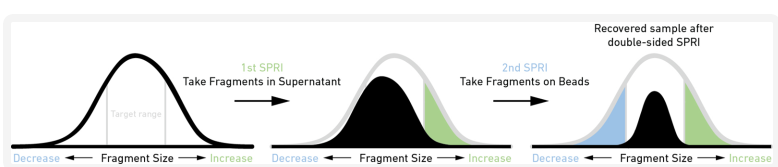
  Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

SPRI beads selectively bind DNA according to the ratio of SPRIselect reagent (beads).

Example Ratio: $= \frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = \mathbf{0.5X}$

Schematic of Double Sided Size Selection



After the first SPRI, supernatant is transferred for a second SPRI while larger fragments are discarded (green). After the second SPRI, fragments on beads are eluted and kept while smaller fragments are discarded (blue). Final sample has a tight fragment size distribution with reduced overall amount (black).

Tutorial — Double Sided Size Selection

1. First SPRIselect: Add **50 µl** SPRIselect reagent to **100 µl** sample (**0.5X**).

Ratio: $= \frac{\text{Volume of SPRIselect reagent added to the sample}}{\text{Volume of DNA sample}} = \frac{50\ \mu l}{100\ \mu l} = 0.5X$

2. Second SPRIselect: Add **30 µl** SPRIselect reagent to supernatant from step a (**0.8X**).

Ratio: $= \frac{\text{Total Volume of reagent added to the sample (step a + b)}}{\text{Original Volume of DNA sample}} = \frac{50\ \mu l + 30\ \mu l}{100\ \mu l} = \mathbf{0.8X}$

Enzymatic Fragmentation

- Ensure enzymatic fragmentation reactions are prepared on ice and then loaded into a thermal cycler pre-cooled to 4°C prior to initiating the Fragmentation, End Repair, and A-tailing incubation steps.

Sample Indices in Sample Index PCR

- Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.
- Each well in the Single Index plate N, Set A contains a unique mix of 4 oligos.
- Each well in the Dual Index Plate TT Set A contains a unique i7 and a unique i5 oligonucleotide.
- Verify and use the specified index plate only. DO NOT use the plates interchangeably.

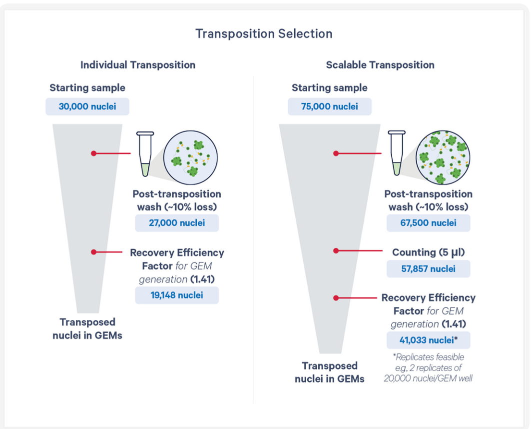
Transposition Selection Guidelines

Transposition Selection Guidelines

The GEM-X Epi Multiome assay accommodates a wide range of nuclei inputs, supporting both Individual Transposition (input ≤30,000 for 500-20,000 Targeted Nuclei Recovery from GEMs) & Scalable Transposition (input ≥30,000 for 20,000–75,000 Targeted Nuclei Recovery from Transposition). Both Individual and Scalable Transposition workflows are similar with few key differences summarized below along with an example. Choose one based on sample and experimental goals.

Parameters	Individual Transposition	Scalable Transposition
Starting nuclei input	Lower (≤30,000) Individual Transposition – Nuclei Input Calculator on page 41	Higher (≥30,000) Scalable Transposition – Nuclei Input Calculator on page 48
Targeted nuclei recovery	500-20,000 *from Transposition + GEM Generation*	20,000-75,000 *from Transposition*
Transposed nuclei resuspension volume	25 µl	35 µl
Transposed nuclei concentration	Not determined	Determined (using 5 µl nuclei suspension)
Execution	Individual Transposition on page 38	Scalable Transposition on page 45
GEM-X Chip loading	25 µl Transposed nuclei	Volume based on adjusting nuclei concentration
Replicates during GEM Generation	Not feasible	Feasible

The workflow example illustrates two different nuclei input amounts. Some nuclei loss is expected during the post transposition wash step. This example assumes ~10% nuclei loss but this will vary between users and starting sample.



Step 1:

Individual Transposition

Choose either Step 1 or Step 2 based on Transposition Selection Guidelines on page 36

1.0 Get Started 39

Individual Transposition – Nuclei Input Calculator 41

1.1 Prepare Transposition Mix 43

1.2 Isothermal Incubation 43

1.3 Post Transposition Wash 44

1.0 Get Started

Item			10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature					
☐	●	ATAC Buffer C	2001581	Thaw, vortex, centrifuge briefly.	−20°C
☐	●	20X Nuclei Buffer *Concentrated 20X stock; dilute 1:20 in nuclease-free water before use. (See Prepare Diluted Nuclei Buffer)*	2000207	Thaw. Vortex, centrifuge briefly.	−20°C
☐	○	Reducing Agent B	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐		Nuclease-free Water	-	-	-
Place on Ice					
☐	⚠	RNase Inhibitor	2001488	-	−20°C
		For Post Transposition Wash Buffer, use **ONLY** PN-2001488 For Diluted Nuclei Buffer, use PN-2001488 or equivalent			
☐	●	ATAC Enzyme B	2001582/ 2001583	Centrifuge briefly, pipette mix	−20°C
☐		Nuclei* in Diluted Nuclei Buffer *(See below to Prepare Diluted Nuclei Buffer)*			

*Refer to protocols for Nuclei Isolation for ATAC + Gene Expression (CG000365, CG000375, CG000505). Adhering to these protocols is critical for optimal assay performance.

Item	10x PN	Preparation & Handling	Storage

Prepare Diluted Nuclei Buffer *Maintain at 4°C*

The use of the Tris-based Diluted Nuclei Buffer for nuclei suspension is critical for optimal assay performance. The composition of the Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps. Suspension of nuclei in a different buffer may not be compatible with the downstream protocol steps.

☐

		Stock	Final	Volume (µl)
●	20X Nuclei Buffer (PN-2000207; equilibrated to room temp.)	20X	1X	50 µl
○	Reducing Agent B (PN-2000087; equilibrated to room temp.)	1,000 mM	1 mM	1 µl
	RNase Inhibitor (Use PN-2001488 or equivalent. Confirm vendor-specific stock concentration)	40 U/µl	1 U/µl	25 µl
	Nuclease-free Water	-	-	924 µl
	Total	**-**	**-**	**1000 µl**

Individual Transposition – Nuclei Input Calculator

Targeted Nuclei Recovery from Individual Transposition followed by GEM Generation: 500-20,000

Determine Nuclei Stock and Diluted Nuclei Buffer* volumes (total 15 µl) for the Transposition Mix using this guidance:

- Based on the starting Nuclei Stock Concentration, locate the corresponding row in the table. Identify the cell with pre-calculated volumes that align with the Targeted Nuclei Recovery. These values already account for a Recovery Efficiency Factor of 1.41 from GEM Generation.
- For concentrations not listed in the table, use the formula provided after the tables to calculate custom volumes.

Higher nuclei concentrations result in smaller pipetting volumes, which may increase variability.

**Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water) as specified in the previous page.*

Nuclei Stock (µl) | Diluted Nuclei Buffer (µl)

Nuclei Stock Concentration (Nuclei/µl)	Targeted Nuclei Recovery (from Transposition + GEM Generation)										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
1000	0.7	1.4	2.8	4.2	5.6	7.1	8.5	9.9	11.3	12.7	14.1
	14.3	13.6	12.2	10.8	9.4	8.0	6.5	5.1	3.7	2.3	0.9
2000	0.4	0.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7.1
	14.6	14.3	13.6	12.9	12.2	11.5	10.8	10.1	9.4	8.7	8.0
3000	0.2	0.5	0.9	1.4	1.9	2.4	2.8	3.3	3.8	4.2	4.7
	14.8	14.5	14.1	13.6	13.1	12.7	12.2	11.7	11.2	10.8	10.3
4000	0.2	0.4	0.7	1.1	1.4	1.8	2.1	2.5	2.8	3.2	3.5
	14.8	14.6	14.3	13.9	13.6	13.2	12.9	12.5	12.2	11.8	11.5
5000	0.1	0.3	0.6	0.8	1.1	1.4	1.7	2.0	2.3	2.5	2.8
	14.9	14.7	14.4	14.2	13.9	13.6	13.3	13.0	12.7	12.5	12.2
6000	0.1	0.2	0.5	0.7	0.9	1.2	1.4	1.6	1.9	2.1	2.4
	14.9	14.8	14.5	14.3	14.1	13.8	13.6	13.4	13.1	12.9	12.7
7000	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
	14.9	14.8	14.6	14.4	14.2	14.0	13.8	13.6	13.4	13.2	13.0
8000	0.1	0.2	0.4	0.5	0.7	0.9	1.1	1.2	1.4	1.6	1.8
	14.9	14.8	14.6	14.5	14.3	14.1	13.9	13.8	13.6	13.4	13.2
9000	0.1	0.2	0.3	0.5	0.6	0.8	0.9	1.1	1.3	1.4	1.6
	14.9	14.8	14.7	14.5	14.4	14.2	14.1	13.9	13.7	13.6	13.4
10000	0.1	0.1	0.3	0.4	0.6	0.7	0.8	1.0	1.1	1.3	1.4
	14.9	14.9	14.7	14.6	14.4	14.3	14.2	14.0	13.9	13.7	13.6
11000	0.1	0.1	0.3	0.4	0.5	0.6	0.8	0.9	1.0	1.2	1.3
	14.9	14.9	14.7	14.6	14.5	14.4	14.2	14.1	14.0	13.8	13.7
12000	0.1	0.1	0.2	0.4	0.5	0.6	0.7	0.8	0.9	1.1	1.2
	14.9	14.9	14.8	14.6	14.5	14.4	14.3	14.2	14.1	13.9	13.8

Volumes that would exceed the allowable buffer volume in each reaction

Low transfer volume that may result in higher nuclei load variability

Nuclei Stock (µl) | Diluted Nuclei Buffer (µl)

Nuclei Stock Concentration (Nuclei/µl)	Targeted Nuclei Recovery *(from Transposition + GEM Generation)*									
	11000	12000	13000	14000	15000	16000	17000	18000	19000	20000
1000										
2000	7.8	8.5	9.2	9.9	10.6	11.3	12.0	12.7	13.4	14.1
	7.2	6.5	5.8	5.1	4.4	3.7	3.0	2.3	1.6	0.9
3000	5.2	5.6	6.1	6.6	7.1	7.5	8.0	8.5	8.9	9.4
	9.8	9.4	8.9	8.4	8.0	7.5	7.0	6.5	6.1	5.6
4000	3.9	4.2	4.6	4.9	5.3	5.6	6.0	6.3	6.7	7.1
	11.1	10.8	10.4	10.1	9.7	9.4	9.0	8.7	8.3	8.0
5000	3.1	3.4	3.7	3.9	4.2	4.5	4.8	5.1	5.4	5.6
	11.9	11.6	11.3	11.1	10.8	10.5	10.2	9.9	9.6	9.4
6000	2.6	2.8	3.1	3.3	3.5	3.8	4.0	4.2	4.5	4.7
	12.4	12.2	11.9	11.7	11.5	11.2	11.0	10.8	10.5	10.3
7000	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
	12.8	12.6	12.4	12.2	12.0	11.8	11.6	11.4	11.2	11.0
8000	1.9	2.1	2.3	2.5	2.6	2.8	3.0	3.2	3.3	3.5
	13.1	12.9	12.7	12.5	12.4	12.2	12.0	11.8	11.7	11.5
9000	1.7	1.9	2.0	2.2	2.4	2.5	2.7	2.8	3.0	3.1
	13.3	13.1	13.0	12.8	12.7	12.5	12.3	12.2	12.0	11.9
10000	1.6	1.7	1.8	2.0	2.1	2.3	2.4	2.5	2.7	2.8
	13.4	13.3	13.2	13.0	12.9	12.7	12.6	12.5	12.3	12.2
11000	1.4	1.5	1.7	1.8	1.9	2.1	2.2	2.3	2.4	2.6
	13.6	13.5	13.3	13.2	13.1	12.9	12.8	12.7	12.6	12.4
12000	1.3	1.4	1.5	1.6	1.8	1.9	2.0	2.1	2.2	2.4
	13.7	13.6	13.5	13.4	13.2	13.1	13.0	12.9	12.8	12.7

Volumes that would exceed the allowable buffer volume in each reaction

Low transfer volume that may result in higher nuclei load variability

Individual Transposition — Formula for Nuclei Stock & Diluted Nuclei Buffer volumes

$$\text{Nuclei Stock Volume (µl)} = \frac{\text{Targeted Nuclei Recov. (GEM Gen.)} \times 1.41 \text{ (Recovery Efficiency Factor-GEM Gen.)}}{\text{Nuclei Stock Concentration (nuclei/µl)}}$$

Diluted Nuclei Buffer Vol. (µl) = 15 µl - Nuclei Stock Volume µl

Example Calculation

$$\text{Nuclei Stock Volume (µl)} = \frac{20000 \times 1.41}{8000} = \mathbf{3.5\ µl}$$

Diluted Nuclei Buffer Vol. (µl) = 15 µl - 3.5 µl = **11.5 µl**

1.1 Prepare Transposition Mix

a. Prepare Transposition Mix on ice. Pipette mix 10x and centrifuge briefly.

	Transposition Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
●	ATAC Buffer C	2001581	5.0	22.0	44.0
●	ATAC Enzyme B	2001582/ 2001583	5.0	22.0	44.0
	Total	-	**10.0**	**44.0**	**88.0**

b. Add **10 µl** Transposition Mix to a tube of a PCR 8-tube strip for each sample. Centrifuge briefly and maintain on ice.

c. Refer to Individual Transposition – Nuclei Input Calculator on page 41 to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of **15 µl**.

d. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix 10x (pipette set to 10 µl). Centrifuge briefly.

e. Gently pipette mix the Nuclei Stock. Add the calculated volume of the Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 5x (pipette set to 20 µl). DO NOT centrifuge.

1.2 Isothermal Incubation

a. Incubate in a thermal cycler using the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
50°C	25 µl	30 min
Step	**Temperature**	**Time**
Incubate	37°C	00:30:00 *(for cell suspensions like PBMC)* 00:60:00 *(for tissue)*
Hold	4°C	00:00:20

During the incubation, prepare Transposition Wash Buffer as outlined in Post Transposition Wash on the next page.

b. **Immediately** proceed to the next step.

1.3 GEM-X Post Transposition Wash

a. Prepare Transposition Wash Buffer on ice. Pipette mix 10x and centrifuge briefly.

	Transposition Wash Buffer ***Add reagents in the order listed***	**PN**	**1X (µl)**	**4X + 10% (µl)**	**8X + 10% (µl)**
	20X Nuclei Buffer	2000207	9.0	39.6	79.2
	Reducing Agent B	2000087	0.4	1.8	3.5
	RNase Inhibitor (use ONLY PN-2001488)	2001488	7.6	33.4	66.9
	Nuclease-Free Water	-	283.0	1245.2	2490.4
	Total	-	**300**	**1,320**	**2,640**

b. Add **200 µl** Transposition Wash Buffer to each sample. Maintain on ice.

c. Gently pipette mix 10x.

d. Place the tube strips on plates and use plate adapters to centrifuge at **500 rcf** for **5 min** at **4°C**.

Using a swinging bucket centrifuge can increase nuclei recovery during centrifugation.

During centrifugation, Master Mix may be prepared as outlined in *Prepare Master Mix on page 54*.

e. Using a pipette tip slightly submerged in the supernatant, start aspirating **200 µl** supernatant. When aspirating, continue to keep the pipette tip slightly submerged by moving it down the tube along with the supernatant, leaving behind ~25 µl supernatant in the original tube along with the pellet. The pellet may not be always visible.

If uncertain about the volume remaining in the tube, add 25 µl water/PBS to an empty tube and use it for a visual estimation.

f. Resuspend the pellet in the residual 25 µl supernatant (gently pipette mix 20x) and **immediately** proceed to GEM Generation.

DO NOT count nuclei.

Step 2:

Scalable Transposition

Choose either Step 1 or Step 2 based on Transposition Selection Guidelines on page 36

2.0 Get Started 46

Scalable Transposition – Nuclei Input Calculator 48

2.1 Prepare Transposition Mix 50

2.2 Isothermal Incubation 50

2.3 Post Transposition Wash 51

2.0 Get Started

Item			10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature					
☐	●	ATAC Buffer C	2001581	Thaw, vortex, centrifuge briefly.	−20°C
☐	●	20X Nuclei Buffer *Concentrated 20X stock; dilute 1:20 in nuclease-free water before use. (See Prepare Diluted Nuclei Buffer)*	2000207	Thaw. Vortex, centrifuge briefly.	−20°C
☐	○	Reducing Agent B	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐		Nuclease-free Water	-	-	-
Place on Ice					
☐	⚠	RNase Inhibitor	2001488	-	−20°C
		For Post Transposition Wash Buffer, use **ONLY** PN-2001488 For Diluted Nuclei Buffer, use PN-2001488 or equivalent			
☐	●	ATAC Enzyme B	2001582/ 2001583	Centrifuge briefly, pipette mix	−20°C
☐		Nuclei* in Diluted Nuclei Buffer *(See below to Prepare Diluted Nuclei Buffer)*			

*Refer to protocols for Nuclei Isolation for ATAC + Gene Expression (CG000365, CG000375, CG000505). Adhering to these protocols is critical for optimal assay performance.

Scalable Transp.

Item	10x PN	Preparation & Handling	Storage
Prepare Diluted Nuclei Buffer *Maintain at 4°C*			

The use of the Tris-based Diluted Nuclei Buffer for nuclei suspension is critical for optimal assay performance. The composition of the Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps. Suspension of nuclei in a different buffer may not be compatible with the downstream protocol steps.

☐			Stock	Final	Volume (µl)
	●	20X Nuclei Buffer (PN-2000207; equilibrated to room temp.)	20X	1X	50 µl
	○	Reducing Agent B (PN-2000087; equilibrated to room temp.)	1,000 mM	1 mM	1 µl
		RNase Inhibitor (Use PN-2001488 or equivalent. Confirm vendor-specific stock concentration)	40 U/µl	1 U/µl	25 µl
		Nuclease-free Water	-	-	924 µl
		Total	**-**	**-**	**1000 µl**

Scalable Transp.

Scalable Transposition – Nuclei Input Calculator

Targeted Nuclei Recovery from Transposition: 20,000-75,000

Determine Nuclei Stock and Diluted Nuclei Buffer* volumes (total 15 µl) for the Transposition Mix using this guidance:

- Based on the starting Nuclei Stock Concentration, locate the corresponding row in the table. Identify the exact cell with pre-calculated volumes based on the Targeted Nuclei Recovery. These values already account for a Recovery Efficiency Factor of 1.0 from Transposition.
- For concentrations not listed in the table, use the formula provided after the table to calculate custom volumes.

Higher nuclei concentrations result in smaller pipetting volumes, which may increase variability.

**Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water) as specified in the previous page.*

Nuclei Stock (µl) | Diluted Nuclei Buffer (µl)

Nuclei Stock Concentration (Nuclei/µl)	Targeted Nuclei Recovery (from Transposition)											
	20000	25000	30000	35000	40000	45000	50000	55000	60000	65000	70000	75000
1000												
2000	10.0											
	5.0											
3000	6.7	8.3	10.0									
	8.3	6.7	5.0									
4000	5.0	6.3	7.5	8.8	10.0							
	10.0	8.8	7.5	6.3	5.0							
5000	4.0	5.0	6.0	7.0	8.0	9.0	10.0					
	11.0	10.0	9.0	8.0	7.0	6.0	5.0					
6000	3.3	4.2	5.0	5.8	6.7	7.5	8.3	9.2	10.0			
	11.7	10.8	10.0	9.2	8.3	7.5	6.7	5.8	5.0			
7000	2.9	3.6	4.3	5.0	5.7	6.4	7.1	7.9	8.6	9.3	10.0	
	12.1	11.4	10.7	10.0	9.3	8.6	7.9	7.1	6.4	5.7	5.0	
8000	2.5	3.1	3.8	4.4	5.0	5.6	6.3	6.9	7.5	8.1	8.8	9.4
	12.5	11.9	11.3	10.6	10.0	9.4	8.8	8.1	7.5	6.9	6.3	5.6
9000	2.2	2.8	3.3	3.9	4.4	5.0	5.6	6.1	6.7	7.2	7.8	8.3
	12.8	12.2	11.7	11.1	10.6	10.0	9.4	8.9	8.3	7.8	7.2	6.7
10000	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
	13.0	12.5	12.0	11.5	11.0	10.5	10.0	9.5	9.0	8.5	8.0	7.5
11000	1.8	2.3	2.7	3.2	3.6	4.1	4.5	5.0	5.5	5.9	6.4	6.8
	13.2	12.7	12.3	11.8	11.4	10.9	10.5	10.0	9.5	9.1	8.6	8.2
12000	1.7	2.1	2.5	2.9	3.3	3.8	4.2	4.6	5.0	5.4	5.8	6.3
	13.3	12.9	12.5	12.1	11.7	11.3	10.8	10.4	10.0	9.6	9.2	8.8

Volumes that would exceed the allowable buffer volume in each reaction

Low transfer volume that may result in higher nuclei load variability

Scalable Transposition — Formula for Nuclei Stock & Diluted Nuclei Buffer volumes

$$\text{Nuclei Stock Volume } (\mu l) = \frac{\text{Nuclei Input for Transp. x 1.0 (Recovery Efficiency Factor-Scal. Transp.)}}{\text{Nuclei Stock Concentration (nuclei/}\mu l\text{)}}$$

$$\text{Diluted Nuclei Buffer Vol. } (\mu l) = 15\ \mu l - \text{Nuclei Stock Volume } \mu l$$

After Scalable Transposition, nuclei concentration is determined and the transposed nuclei input volume for GEM Generation is adjusted based on Targeted Nuclei Recovery from GEMs, factoring in Recovery Efficiency Factor for GEM Generation (1.41), as specified in *Transposed Nuclei Suspension Volume Calculator Table for GEM Generation on page 56*

Example Calculation

$$\text{Nuclei Stock Volume } (\mu l) = \frac{75000 \text{ X } 1.0}{8000} = \mathbf{9.4\ \mu l}$$

$$\text{Diluted Nuclei Buffer Vol. } (\mu l) = 15\ \mu l - 9.4\ \mu l = \mathbf{5.6\ \mu l}$$

Scalable Transp.

2.1 Prepare Transposition Mix

a. Prepare Transposition Mix on ice. Pipette mix 10x and centrifuge briefly.

	Transposition Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
●	ATAC Buffer C	2001581	5.0	22.0	44.0
●	ATAC Enzyme B	2001582/ 2001583	5.0	22.0	44.0
	Total	-	**10.0**	**44.0**	**88.0**

b. Add **10 µl** Transposition Mix to a tube of a PCR 8-tube strip for each sample. Centrifuge briefly and maintain on ice.

c. Scalable Transposition: Refer to Scalable Transposition – Nuclei Input Calculator on page 48 to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of **15 µl**.

d. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix 10x (pipette set to 10 µl). Centrifuge briefly.

e. Gently pipette mix the Nuclei Stock. Add the calculated volume of the Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 5x (pipette set to 20 µl). DO NOT centrifuge.

2.2 Isothermal Incubation

a. Incubate in a thermal cycler using the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
50°C	25 µl	30 min
Step	**Temperature**	**Time**
Incubate	37°C	00:30:00 *(for cell suspensions like PBMC)* 00:60:00 *(for tissue)*
Hold	4°C	00:00:20

During the incubation, prepare Transposition Wash Buffer as outlined in Post Transposition Wash on the next page.

b. **Immediately** proceed to the next step.

2.3 GEM-X Post Transposition Wash

a. Prepare Transposition Wash Buffer on ice. Pipette mix 10x and centrifuge briefly.

	Transposition Wash Buffer ***Add reagents in the order listed***	**PN**	**1X (µl)**	**4X + 10% (µl)**	**8X + 10% (µl)**
●	20X Nuclei Buffer	2000207	9.0	39.6	79.2
○	Reducing Agent B	2000087	0.4	1.8	3.5
●	RNase Inhibitor (use ONLY PN-2001488)	2001488	7.6	33.4	66.9
	Nuclease-Free Water	-	283.0	1245.2	2490.4
	Total	-	**300**	**1,320**	**2,640**

b. Add **200 µl** Transposition Wash Buffer to each sample. Maintain on ice.

c. Gently pipette mix 10x.

Remaining Transposition Wash Buffer can be maintained on ice for dilutions in GEM Generation step.

d. Place the tube strips on plates and use plate adapters to centrifuge at **500 rcf** for **5 min** at **4°C**.

Using a swinging bucket centrifuge can increase nuclei recovery during centrifugation. During centrifugation, Master Mix may be prepared as outlined in *Prepare Master Mix on page 54*.

e. Using a pipette tip slightly submerged in the supernatant, start aspirating **190 µl** supernatant. When aspirating, continue to keep the pipette tip slightly submerged by moving it down the tube along with the supernatant, leaving behind ~35 µl supernatant in the original tube along with the pellet. The pellet may not be always visible.

If uncertain about the volume remaining in the tube, add 35 µl water/PBS to an empty tube and use it for a visual estimation.

f. Resuspend the pellet in the residual 35 µl supernatant (pipette mix 20x) and maintain the tube on ice.

Use 5 µl nuclei suspension to count and determine nuclei concentration and then proceed to GEM Generation.

5-10 µl may be used for counting depending on specific sample and counting method. For accurate nuclei counting guidelines, consult sample preparation protocols CG000365 Rev E or CG000505 Rev B.

Step 3:

GEM Generation & Barcoding

3.0 Get Started 53

3.1 Prepare Master Mix 54

Transposed Nuclei Suspension Volume Calculator Table for GEM Generation 56

3.2 Load GEM-X Chip 58

3.3 Run the Chromium X Series Instrument (X/iX) 61

3.4 Transfer GEMs 61

3.5 GEM Incubation 63

3.6 Quenching Reaction 63

3.0 Get Started

Firmware version 2.0.0 or higher is required in the Chromium X Series instrument to run the GEM-X chips.

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature					
☐		GEM-X Multiome Gel Beads	2001514	Equilibrate to room temperature 30 min before loading the chip.	−80°C
☐	●	Template Switch Oligo B	2001027	Centrifuge briefly, resuspend in **52.8 µl** Low TE Buffer. Vortex 15 sec at maximum speed, centrifuge briefly. Resuspended solution can be used immediately. After resuspension, store at **−80°C.** Thaw at room temperature for ≥ 30 minutes in subsequent uses.	−20°C
☐	○	Reducing Agent B	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐	●	Barcoding Reagent C	2001558	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐	●	Quenching Agent	2001679	Equilibrate to room temperature during GEM Incubation step	−20°C
Place on Ice					
☐	●	Barcoding Enzyme B	2001562/ 2001561	Centrifuge briefly. Maintain on ice. Gently pipette mix right before use. Store at −20°C immediately after use.	−20°C
Obtain					
☐	●	Partitioning Oil B	2001213	-	Ambient
☐		Low TE Buffer	-	Manufacturer's recommendations.	-
☐		GEM-X MO Chip	2001485	See Tips & Best Practices.	Ambient
☐		10x Gasket	3000656	See Tips & Best Practices.	Ambient
☐		10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
☐		Chromium X Series Chip Holder	3000598	See Tips & Best Practices	Ambient
☐		50% glycerol solution *If using <8 reactions*	-	See Tips & Best Practices.	-

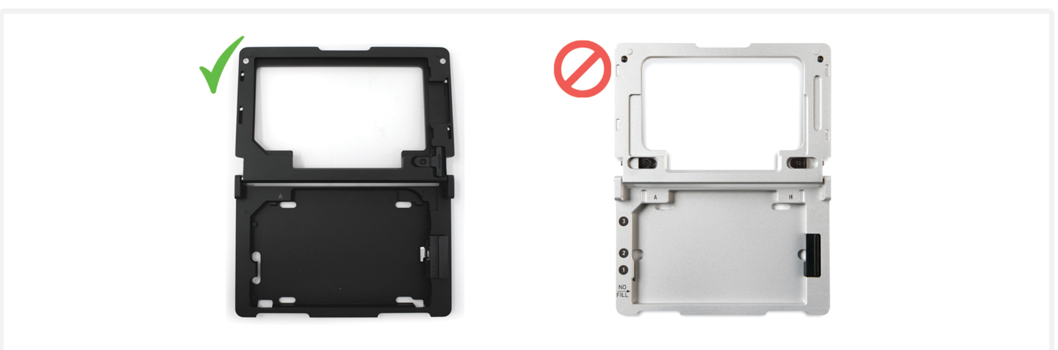
3.1 Prepare Master Mix

a. Prepare Master Mix on ice. Pipette mix 10x and centrifuge briefly.

	Master Mix *Add reagents in the order listed*	**PN**	**1X (µl)**	**4X + 10% (µl)**	**8X + 10% (µl)**
●	Barcoding Reagent C	2001558	29.5	129.8	259.6
●	Template Switch Oligo B	2001027	1.1	4.8	9.7
○	Reducing Agent B	2000087	1.9	8.3	16.7
●	Barcoding Enzyme B	2001562/ 2001561	7.5	33.0	66.0
	Total	-	**40.0**	**176.0**	**352.0**

GEM-X Assemble GEM-X Chip

GEM-X MO Chip is only compatible with Chromium X Series Chip Holder (PN-3000598). DO NOT use any other holder.



See Tips & Best Practices on page 25 for chip handling instructions.

a. Close the holder lid. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.

b. DO NOT touch the smooth side of the gasket.

c. Open the chip holder.

d. Remove the chip from the sealed bag. Use the chip within ≤ 24 h.

e. Align notch on the chip (upper left corner) and the open holder with the gasket attached.

f. Slide the chip to the left until the chip is inserted under the guide on the holder. Depress the right hand side of the chip until the spring-loaded clip engages.

g. Keep the assembled unit with the attached gasket open until ready for loading and while dispensing reagents into the wells.

h. DO NOT touch the smooth side of the gasket.

i. The assembled chip is ready for loading the indicated reagents. See Load GEM-X Chip on page 58 for reagent volumes and loading order.

j. After loading reagents, close the chip holder. DO NOT press down on the top of the gasket.

For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 & 3.

DO NOT load reagents in the top row labeled NO FILL.

Transposed Nuclei Suspension Volume Calculator Table for GEM Generation

For Scalable Transposition samples, refer to the table below to execute Step 3.2c. These values apply only to samples where nuclei concentration was determined after the Post Transposition Wash.

DO NOT add Transposition Wash Buffer directly to the nuclei suspension. Add Transposition Wash Buffer to the Master Mix. See step 3.2c.

Transposed Nuclei (µl) | Transposition Wash Buffer (µl)

Transposed Nuclei Conc. *after Post Transp. Wash* **(Nuclei/µl)**	**Targeted Nuclei Recovery**										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	7.1	14.1									
	18.0	10.9									
200	3.5	7.1	14.1	21.2							
	21.5	18.0	10.9	3.9							
300	2.4	4.7	9.4	14.1	18.8	23.5					
	22.7	20.3	15.6	10.9	6.2	1.5					
400	1.8	3.5	7.1	10.6	14.1	17.6	21.2				
	23.2	21.5	18.0	14.4	10.9	7.4	3.9				
500	1.4	2.8	5.6	8.5	11.3	14.1	16.9	19.7	22.6		
	23.6	22.2	19.4	16.5	13.7	10.9	8.1	5.3	2.4		
600	1.2	2.4	4.7	7.1	9.4	11.8	14.1	16.5	18.8	21.2	23.5
	23.8	22.7	20.3	18.0	15.6	13.3	10.9	8.6	6.2	3.9	1.5
700	1.0	2.0	4.0	6.0	8.1	10.1	12.1	14.1	16.1	18.1	20.1
	24.0	23.0	21.0	19.0	16.9	14.9	12.9	10.9	8.9	6.9	4.9
800	0.9	1.8	3.5	5.3	7.1	8.8	10.6	12.3	14.1	15.9	17.6
	24.1	23.2	21.5	19.7	18.0	16.2	14.4	12.7	10.9	9.1	7.4
900	0.8	1.6	3.1	4.7	6.3	7.8	9.4	11.0	12.5	14.1	15.7
	24.2	23.4	21.9	20.3	18.7	17.2	15.6	14.0	12.5	10.9	9.3
1000	0.7	1.4	2.8	4.2	5.6	7.1	8.5	9.9	11.3	12.7	14.1
	24.3	23.6	22.2	20.8	19.4	18.0	16.5	15.1	13.7	12.3	10.9
1100	0.6	1.3	2.6	3.8	5.1	6.4	7.7	9.0	10.3	11.5	12.8
	24.4	23.7	22.4	21.2	19.9	18.6	17.3	16.0	14.7	13.5	12.2
1200	0.6	1.2	2.4	3.5	4.7	5.9	7.1	8.2	9.4	10.6	11.8
	24.4	23.8	22.7	21.5	20.3	19.1	18.0	16.8	15.6	14.4	13.3
1300	0.5	1.1	2.2	3.3	4.3	5.4	6.5	7.6	8.7	9.8	10.8
	24.5	23.9	22.8	21.7	20.7	19.6	18.5	17.4	16.3	15.2	14.2
1400	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1
	24.5	24.0	23.0	22.0	21.0	20.0	19.0	18.0	16.9	15.9	14.9
1500	0.5	0.9	1.9	2.8	3.8	4.7	5.6	6.6	7.5	8.5	9.4
	24.5	24.1	23.1	22.2	21.2	20.3	19.4	18.4	17.5	16.5	15.6
1600	0.4	0.9	1.8	2.6	3.5	4.4	5.3	6.2	7.1	7.9	8.8
	24.6	24.1	23.2	22.4	21.5	20.6	19.7	18.8	18.0	17.1	16.2
1700	0.4	0.8	1.7	2.5	3.3	4.1	5.0	5.8	6.6	7.5	8.3
	24.6	24.2	23.3	22.5	21.7	20.9	20.0	19.2	18.4	17.5	16.7
1800	0.4	0.8	1.6	2.4	3.1	3.9	4.7	5.5	6.3	7.1	7.8
	24.6	24.2	23.4	22.7	21.9	21.1	20.3	19.5	18.7	18.0	17.2
1900	0.4	0.7	1.5	2.2	3.0	3.7	4.5	5.2	5.9	6.7	7.4
	24.6	24.3	23.5	22.8	22.0	21.3	20.5	19.8	19.1	18.3	17.6
2000	0.4	0.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7.1
	24.6	24.3	23.6	22.9	22.2	21.5	20.8	20.1	19.4	18.7	18.0

Volumes that would exceed the allowable buffer volume in each reaction

Low transfer volume that may result in higher cell load variability

Optimal range of transposed nuclei concentration to maximize the likelihood of achieving the desired nuclei recovery target (500-10,000 nuclei)

Optimal range of transposed nuclei concentration to maximize the likelihood of achieving the desired nuclei recovery target (10,000-20,000 nuclei)

Transposed Nuclei (µl) | Transposition Wash Buffer (µl)

Transposed Nuclei Conc. *after Post Transp. Wash* (Nuclei/µl)	Targeted Nuclei Recovery										
	10000	11000	12000	13000	14000	15000	16000	17000	18000	19000	20000
100											
200											
300											
400											
500											
600	23.5										
	1.5										
700	20.1	22.2	24.2								
	4.9	2.8	0.8								
800	17.6	19.4	21.2	22.9	24.7						
	7.4	5.6	3.9	2.1	0.3						
900	15.7	17.2	18.8	20.4	21.9	23.5					
	9.3	7.8	6.2	4.6	3.1	1.5					
1000	14.1	15.5	16.9	18.3	19.7	21.2	22.6	24.0			
	10.9	9.5	8.1	6.7	5.3	3.9	2.4	1.0			
1100	12.8	14.1	15.4	16.7	17.9	19.2	20.5	21.8	23.1	24.4	
	12.2	10.9	9.6	8.3	7.1	5.8	4.5	3.2	1.9	0.6	
1200	11.8	12.9	14.1	15.3	16.5	17.6	18.8	20.0	21.2	22.3	23.5
	13.3	12.1	10.9	9.7	8.6	7.4	6.2	5.0	3.9	2.7	1.5
1300	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.4	19.5	20.6	21.7
	14.2	13.1	12.0	10.9	9.8	8.7	7.6	6.6	5.5	4.4	3.3
1400	10.1	11.1	12.1	13.1	14.1	15.1	16.1	17.1	18.1	19.1	20.1
	14.9	13.9	12.9	11.9	10.9	9.9	8.9	7.9	6.9	5.9	4.9
1500	9.4	10.3	11.3	12.2	13.2	14.1	15.0	16.0	16.9	17.9	18.8
	15.6	14.7	13.7	12.8	11.8	10.9	10.0	9.0	8.1	7.1	6.2
1600	8.8	9.7	10.6	11.5	12.3	13.2	14.1	15.0	15.9	16.7	17.6
	16.2	15.3	14.4	13.5	12.7	11.8	10.9	10.0	9.1	8.3	7.4
1700	8.3	9.1	10.0	10.8	11.6	12.4	13.3	14.1	14.9	15.8	16.6
	16.7	15.9	15.0	14.2	13.4	12.6	11.7	10.9	10.1	9.2	8.4
1800	7.8	8.6	9.4	10.2	11.0	11.8	12.5	13.3	14.1	14.9	15.7
	17.2	16.4	15.6	14.8	14.0	13.3	12.5	11.7	10.9	10.1	9.3
1900	7.4	8.2	8.9	9.6	10.4	11.1	11.9	12.6	13.4	14.1	14.8
	17.6	16.8	16.1	15.4	14.6	13.9	13.1	12.4	11.6	10.9	10.2
2000	7.1	7.8	8.5	9.2	9.9	10.6	11.3	12.0	12.7	13.4	14.1
	18.0	17.2	16.5	15.8	15.1	14.4	13.7	13.0	12.3	11.6	10.9

Volumes that would exceed the allowable buffer volume in each reaction

Low transfer volume that may result in higher cell load variability

Optimal range of transposed nuclei concentration to maximize the likelihood of achieving the desired nuclei recovery target (500-10,000 nuclei)

Optimal range of transposed nuclei concentration to maximize the likelihood of achieving the desired nuclei recovery target (10,000-20,000 nuclei)

3.2 Load GEM-X Chip

- After removing chip from the sealed bag, use in **≤24 h**.
- Open the lid (gasket attached) of the assembled chip and lay flat for loading.
- Ensure that the Gel Beads are properly thawed and ready to use.
- When loading the chip, raising and depressing the pipette plunger should each take **~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

Color Legend
2: Gel beads
1: Sample
3: Oil

The Chromium X/iX (X Series) Chip Holder, Chip Gasket X/iX, and GEM-X chip images shown below are representative and do not show the specific color & label. Chip holder and gasket should be black and blue in color, respectively. Refer to *Assemble GEM-X Chip on page 54* *for details.*

GEM-X MO Chip, gasket attached
Representative chip image

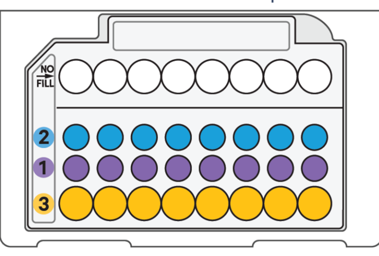
Pipette technique

a. If loading less than 8 samples/chip, add 50% glycerol solution to each unused well in row 1, 2, and 3

- **60 µl** in each unused well in row labeled 1
- **60 µl** in each unused well in row labeled 2
- **250 µl** in each unused well in row labeled 3

DO NOT add 50% glycerol solution to the wells in top row labeled NO FILL.
DO NOT use any substitute for 50% glycerol solution.

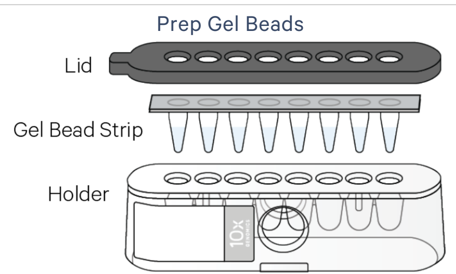
Glycerol
in GEM-X MO Chip



b. Prepare Gel Beads

- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for **~5 sec**.
- Confirm there are no bubbles at the bottom of the tubes & the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.

Prep Gel Beads



c. Prepare Master Mix + Transposed Nuclei

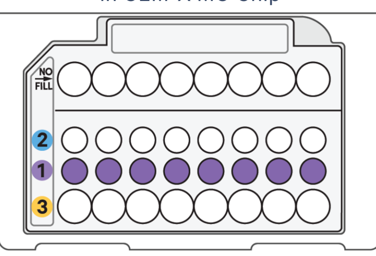
- **Individual Transposition (nuclei not counted after Post Transposition Wash):**
    - Gently pipette mix **25 µl** transposed nuclei in the tube strip and add **40 µl** Master Mix to each tube for a total of **65 µl**, gently pipette mix 10x, and **immediately** proceed to step d.
- **Scalable Transposition (nuclei counted after Post Transposition Wash):**
    - Add **40 µl** Master Mix to each tube of a tube strip.
    - Refer to the Transposed Nuclei Suspension Volume Calculator Table for GEM Generation (page 56)and add appropriate volume of Transposition Wash Buffer to the Master Mix, gently pipette mix 10x, and centrifuge briefly. DO NOT add Transposition Wash Buffer directly to the nuclei suspension.
    - Add appropriate volume of transposed nuclei to the Master Mix. *Gently pipette mix the transposed nuclei before adding. Total volume 65 µl in each tube.*
      Gently pipette mix 10x and **immediately** proceed to step d.

Any remaining Transposed Nuclei may be maintained on **ice** for up to **30 min** if additional rounds of GEM Generation are required.

d. Load Row Labeled 1

- Gently pipette mix the Master Mix + Transposed Nuclei.
- Using the same pipette tip, dispense **60 µl** Master Mix + Transposed Nuclei into the bottom center of each well in **row labeled 1** without introducing bubbles.
- Wait **30 sec**.

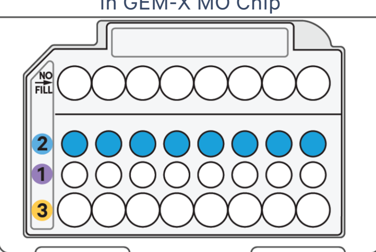
60 µl Master Mix + Cell Suspension in GEM-X MO Chip



e. Load Row Labeled 2

- Puncture the foil seal of the Gel Bead tubes.
- Slowly aspirate **60 µl** Gel Beads.
- Dispense into the bottom center of each well in **row labeled 2** without introducing bubbles.
- Wait **30 sec**.

60 µl Gel Beads in GEM-X MO Chip

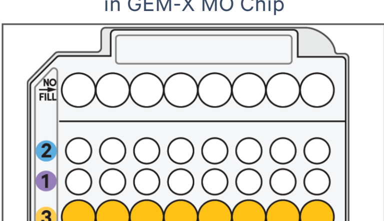


f. Load Row Labeled 3

- Dispense **250 µl** Partitioning Oil B into the wells in **row labeled 3** by pipetting two aliquots of **125 µl** from a reagent reservoir.

Failure to add Partitioning Oil B to the row labeled 3 will prevent GEM generation and can damage the instrument.

250 µl Partitioning Oil B in GEM-X MO Chip



g. Prepare for Run

- Close the lid (gasket already attached). DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket. Keep horizontal to avoid wetting the gasket.
- Keep the chip horizontal and be careful when moving/setting down the chip to avoid wetting the gasket with oil or spilling oil over the outside of the wells.*

GEM-X MO Chip, closed

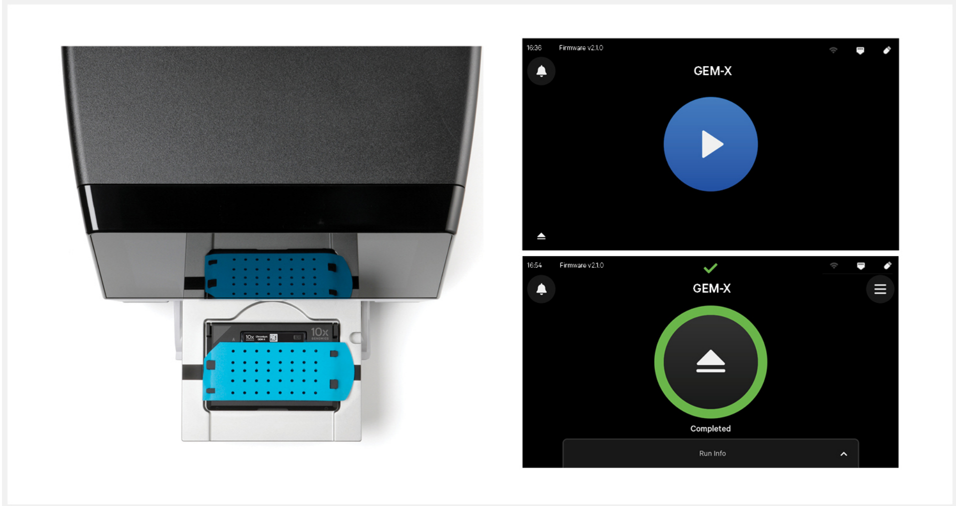
Run the chip in Chromium X series instrument (X/iX) ***immediately*** *after loading the Partitioning Oil B.*

3.3 Run the Chromium X Series Instrument (X/iX)

Firmware Version 2.0.0 or higher is required in the Chromium X Series instrument (X/iX) used for this protocol.

Consult the Chromium X Series (X/iX) User Guide (CG000396) for detailed instrument operation instructions and follow the instrument touchscreen prompts for execution.

a. Press the eject button on the instrument to eject the tray.



If the eject button is not touched within 1 min, tray will close automatically. System requires a few seconds before the tray can be ejected again.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Press the play button.

d. At completion of the run (~6 min), the instrument will chime. **Immediately** proceed to the next step.

3.4 Transfer GEMs

a. Place a tube strip on ice.

b. Press the eject button of the instrument and remove the chip.

c. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees. Be careful when opening the chip holder at 45 degrees to avoid wetting the gasket with oil or spilling oil.

d. Visually compare the remaining volume in rows labeled 1-2. Abnormally high volume in one well relative to other wells may indicate a clog.

e. Take a picture of the GEMs in the pipette tips and/or tube strips.

f. **Slowly** aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top NO FILL row without creating a seal between the tips and the bottom of the wells.

g. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

h. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes.

i. It is recommended to start GEM incubation within **5 min**. If multiple chips are run back-to-back, place the GEMs from the subsequent chips in separate thermal cyclers.

Representative chip image

3.5 GEM Incubation

Use a thermal cycler that can accommodate at least 100 µl volume. A volume of 125 µl is the preferred setting on Bio-Rad C1000 Touch. In alternate thermal cyclers, use highest reaction volume setting.

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
50°C	100 µl	75 min
Step	**Temperature**	**Time**
1	37°C	00:45:00
2	25°C	00:30:00
3	4°C	Hold* (*proceed to next step immediately after run is at 4°C)

Retrieve Quenching Agent (PN-2001679) from -20°C and equilibrate to **room temperature** while the PCR program is running.

*After GEM incubation, proceed **immediately** to the next step.

3.6 Quenching Reaction

a. Add **5 µl** Quenching Agent **directly to** each sample to stop the reaction.

DO NOT pipette mix.

b. Store at **−80°C** for up to **4 weeks**, or proceed to the next step.

Step 4:

Post GEM Incubation Cleanup

4.0 Get Started 65

4.1 Post GEM Incubation Cleanup – Dynabeads 66

4.2 Post GEM Incubation Cleanup – SPRIselect 68

4.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐ ◯	Reducing Agent B	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	−20°C
☐	Nuclease-free Water	-	-	-
☐	Dynabeads MyOne SILANE	2000048	Vortex thoroughly **(≥30 sec)** **immediately** before adding to the mix. If still clumpy, pipette mix to resuspend completely. DO NOT centrifuge before use.	4°C
☐	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
Thaw at 65°C				
☐ ◯	Cleanup Buffer	2000088	Thaw for **10 min** at **65°C** at max speed on a thermomixer. Verify there are no visible crystals. Cool to room temperature.	−20°C
Obtain				
☐	Recovery Agent	220016	-	Ambient
☐	Qiagen Buffer EB	-	Manufacturer's recommendations.	-
☐	Bio-Rad 10% Tween 20	-	Manufacturer's recommendations.	-
☐	10x Magnetic Separator	230003/ 2001212	-	Ambient
☐	Prepare 80% Ethanol *Prepare 10 ml for 8 reactions*	-	Prepare fresh in nuclease-free water.	-

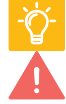
4.1 Post GEM Incubation Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample (Post GEM-RT) at room temperature. *If using sample stored at −80°C, thaw and equilibrate to room temperature for ~15 min before adding the Recovery Agent*. DO NOT pipette mix or vortex the biphasic mixture. Wait **2 min**.

The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

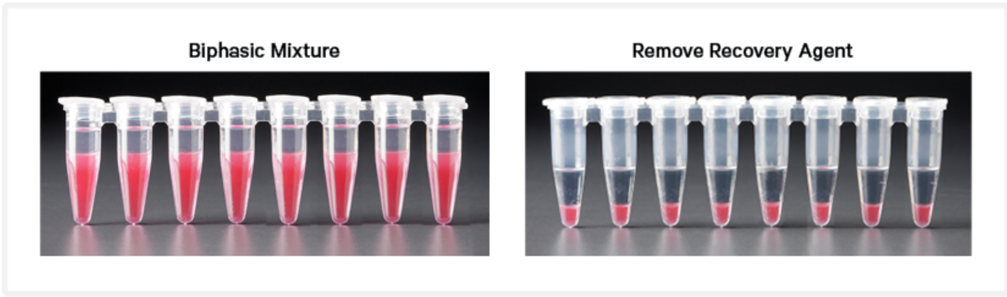
If biphasic separation is incomplete:

- Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim.
- Mix by inverting the capped tube strip 5x, centrifuge briefly, and proceed to step b. DO NOT invert without firmly securing the caps.



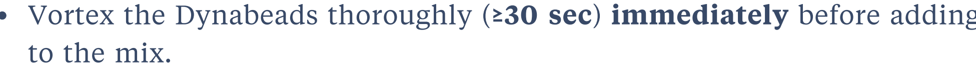
A smaller aqueous phase volume indicates a clog during GEM generation.

b. Slowly remove and discard **125 µl** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.



c. Prepare Dynabeads Cleanup Mix.

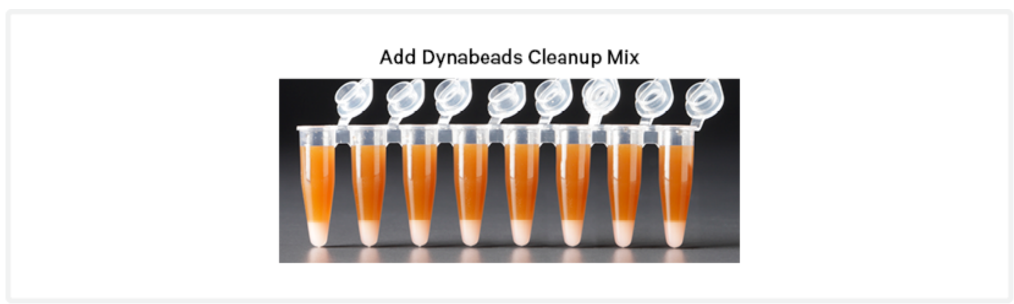
Before using Dynabeads MyOne SILANE to prepare the Dynabeads Cleanup Mix:



- Vortex the Dynabeads thoroughly (≥**30 sec**) **immediately** before adding to the mix.
- Aspirate full liquid volume in the Dynabead tube with a pipette tip to verify that beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before adding to the mix.

	Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	2X + 10% (µl)	4X + 10% (µl)	8X + 10% (µl)
●	Cleanup Buffer	2000088	182	400.5	801	1602
	Dynabeads MyOne SILANE	2000048	8	17.5	35	70
○	Reducing Agent B	2000087	5	11	22	44
	Nuclease-free Water	—	5	11	22	44
	Total		**200**	**440**	**880**	**1760**

d. Vortex and add **200 µl** to each tube. Pipette mix 10x (pipette set to 200 µl). Before adding ensure that there are no clumps.



e. Incubate **10 min** at **room temperature** (keep caps open). Pipette mix again at ~5 min after start of incubation to resuspend settled beads.

f. Prepare Elution Solution I. Vortex and centrifuge briefly.

	Elution Solution I *Add reagents in the order listed*	PN	1X (µl)	10X (µl)
	Buffer EB	—	98	980
	10% Tween 20	—	1	10
○	Reducing Agent B	2000087	1	10
	Total		**100**	**1000**

g. At the end of **10 min** incubation, place on a 10x Magnetic Separator•**High** position (magnet•**High**) until the solution clears.

A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet while on the magnet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the 10x Magnetic Separator•**Low** position (magnet•**Low)**.

n. Remove remaining ethanol. DO NOT dry.

o. Remove from the magnet. Immediately add **50.5 µl** Elution Solution I.

p. Pipette mix (pipette set to 40 µl) without introducing bubbles. Pipette mix 15x. If beads still appear clumpy, continue pipette mixing until fully resuspended.

q. Incubate **1 min** at **room temperature**.

r. Place on the magnet•**Low** until the solution clears.

s. Transfer **50 µl** sample to a new tube strip.

4.2 Post GEM Incubation Cleanup – SPRIselect

a. Vortex the SPRIselect reagent until fully resuspended. Add **90 µl** SPRIselect reagent to each sample. Pipette mix (pipette set to 130 µl) thoroughly.

b. Incubate **5 min** at **room temperature**.

c. Centrifuge briefly. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol.

Residual ethanol can inhibit Pre-Amplification PCR and impact assay performance.

j. Remove the tube strip from the magnet. Air dry for **1 min**. **Immediately** add **46.5 µl** Buffer EB.

k. Pipette mix (pipette set to 45 µl) without introducing bubbles.

l. Incubate **2 min** at **room temperature**.

m. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

n. Transfer **46 µl** sample to a new tube strip.

Residual SPRI beads can inhibit Pre-Amplification PCR and impact assay performance.

Step 5:

Pre Amplification PCR

5.0 Get Started 70

5.1 Prepare Pre Amplification Mix 70

5.2 Pre Amplification PCR 71

5.3 Pre Amplification SPRI Cleanup 71

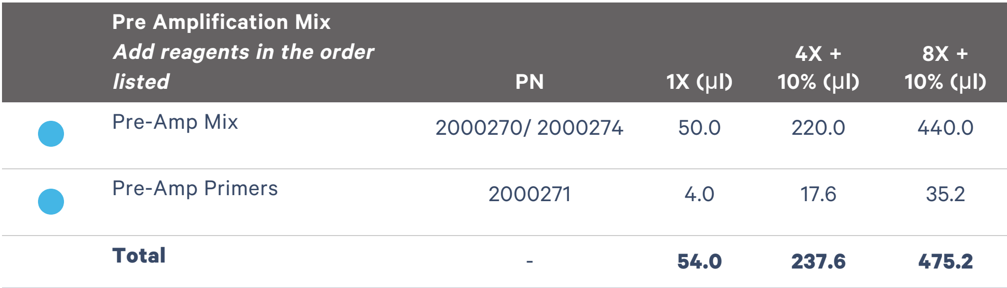
Sample Split Overview 72

5.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐	Pre-Amp Primers	2000271	Vortex, centrifuge briefly.	−20°C
	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
Place on Ice				
☐	Pre-Amp Mix	2000270/ 2000274	Thaw completely on ice. Gently pipette mix, centrifuge briefly.	−20°C
Obtain				
☐	Qiagen Buffer EB	-	-	Ambient
☐	10x Magnetic Separator	230003/ 2001212	See Tips & Best Practices.	Ambient
☐	Prepare 80% Ethanol *Prepare 10 ml for 8 reactions*	-	Prepare fresh.	Ambient

5.1 Prepare Pre Amplification Mix

a. Prepare Pre Amplification Mix on ice. Pipette mix 10x and centrifuge briefly.



	Pre Amplification Mix ***Add reagents in the order listed***	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
	Pre-Amp Mix	2000270/ 2000274	50.0	220.0	440.0
	Pre-Amp Primers	2000271	4.0	17.6	35.2
	Total	-	**54.0**	**237.6**	**475.2**

b. Add **54 µl** Pre Amplification Mix to each sample. Pipette mix and centrifuge briefly.

5.2 Pre Amplification PCR

a. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
105°C	100 µl	30 min
Step	**Temperature**	**Time**
1	72°C	00:05:00
2	98°C	00:03:00
3	98°C	00:00:20
4	63°C	00:00:30
5	72°C	00:01:00 Go to step 3 repeat 5X (Total 6 cycles*)
6	72°C	00:01:00
7	4°C	Hold

**Do not modify cycle number*

b. Store at **4°C** for up to **18 h** or proceed to the next step.

5.3 Pre Amplification SPRI Cleanup

a. Vortex the SPRIselect reagent until fully resuspended. Add **160 µl** SPRIselect reagent to each sample. Pipette mix 15x.

b. Incubate **5 min** at **room temperature**.

c. Centrifuge briefly. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **300 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

h. Remove the ethanol.

i. Centrifuge briefly. Place on the magnet•**Low**.

j. Remove any remaining ethanol.

k. Remove the tube strip from the magnet. Air dry for **2 min**. Add **80.5 µl** Buffer EB.

l. Pipette mix (pipette set to 75 µl) without introducing bubbles.

m. Incubate **2 min** at **room temperature**.

n. Centrifuge briefly. Place on the magnet•**High** until the solution clears.

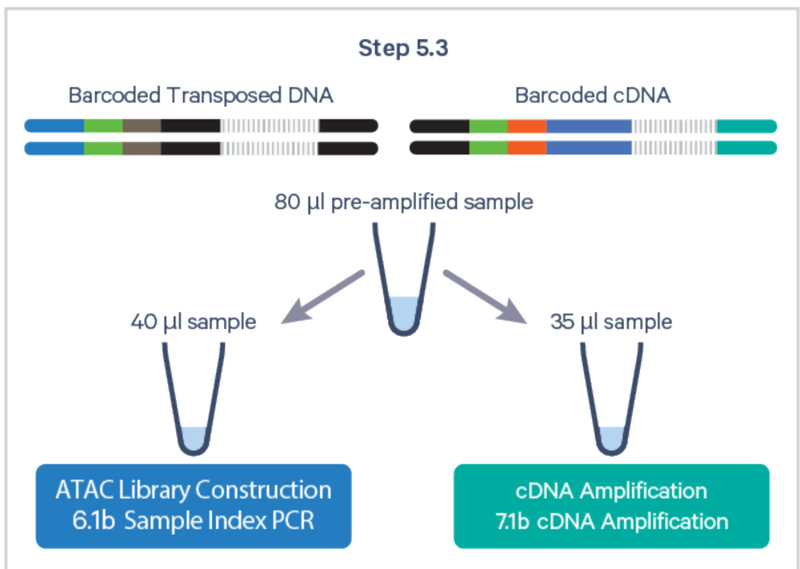
o. Transfer **80 µl** sample to a new tube strip.

p. Store at **4°C** for up to **72 h** or at **−20°C** for long term storage, or proceed to the next step.

Sample Split Overview

- 80 µl pre-amplified, SPRI cleaned sample derived at end of step 5.3 includes barcoded transposed DNA fragments and barcoded cDNA.
- The sample is divided and used as input for two separate steps.
  - i. 40 µl sample is used for ATAC Library Construction (step 6)
  - ii. 35 µl sample is used for cDNA Amplification (step 7).

    The amplified cDNA will be used for Gene Expression Library Construction.



Step 6:

ATAC Library Construction

6.0 Get Started 74

6.1 Sample Index PCR 74

6.2 Post Sample Index Double Sided Size Selection – SPRIselect 75

6.3 Post Library Construction QC 77

6.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐	Sample Index Plate N, Set A	3000427	-	−20°C
☐	Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
☐	Agilent Bioanalyzer DNA kit *(if used for QC)*	-	Manufacturer's recommendations.	-
☐	DNA High Sensitivity Reagent Kit *(if LabChip used for QC)*	-	Manufacturer's recommendations.	-
Place on Ice				
☐ ●	SI-PCR Primer B	2000128	Vortex, centrifuge briefly.	−20°C
☐ ○	Amp Mix	2000047	Gently pipette mix, centrifuge briefly.	−20°C
☐	KAPA Library Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations.	-
Obtain				
☐	Qiagen Buffer EB	-	-	Ambient
☐	10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
☐	Prepare 80% Ethanol *Prepare 10 ml for 8 reactions*	-	Prepare fresh.	Ambient

6.1 Sample Index PCR

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run.

a. Prepare Sample Index PCR Mix. Pipette mix and centrifuge briefly.

	Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○	Amp Mix	2000047	50	220	440
●	SI- PCR Primer B	2000128	7.5	33	66
	Total	**-**	**57.5**	**253**	**506**

b. Transfer **40 µl** pre-amplified sample from step 5.3 to a new tube strip (35 µl of the remaining sample volume will be used for cDNA Amplification). Add

57.5 µl Sample Index PCR Mix to the sample. Pipette mix and centrifuge briefly.

c. Add **2.5 µl** of an individual Sample Index N, Set A to each well. Record assignment. Pipette mix and centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
105°C	100 µl	~30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:00:20 Go to step 2, see table below for # cycles
5	72°C	00:01:00
6	4°C	Hold

The table recommends a starting point for cycle number optimization based on Targeted Nuclei Recovery.

Cycle Number Optimization Table

Targeted Nuclei Recovery	Total Cycles
≤2,000	9
2,001-6,000	8
>6,001	7

e. Store at **4°C** for up to **72 h** or proceed to the next step.

6.2 Post Sample Index Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **60 µl** SPRIselect reagent (0.6X) to each sample. Pipette mix 15x.

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Transfer **150 µl** supernatant to a new strip tube. DO NOT discard the supernatant.

e. Vortex to resuspend SPRIselect reagent. Add **95 µl** SPRIselect reagent (1.55X) to each sample (supernatant). Pipette mix 15x.

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly. Place on the magnet•**Low**.

n. Remove remaining ethanol. Air dry for **2 min**.

o. Remove from the magnet. **Immediately** add **20.5** µl Buffer EB. Pipette mix.

p. Incubate **2 min** at **room temperature**.

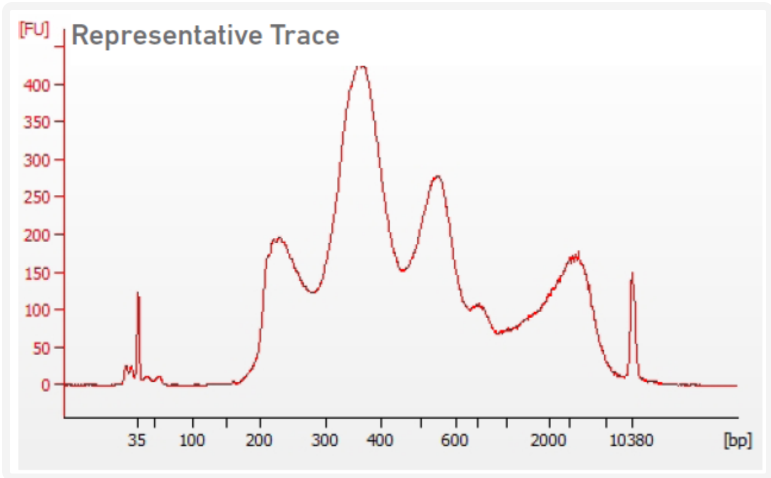
q. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

r. Transfer **20 µl** sample to a new tube strip.

s. Store at **4°C** for up to **72 h** or at **−20°C** for long-term storage.

6.3 Post Library Construction QC

a. Run 1 µl sample at 1:2 dilution on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size. Select the region between 175-1000 bp to determine average size of ATAC library. Lower molecular weight product (≤ 150 bp) and/or a high molecular weight product (~2,000 bp) may be present. This does not affect sequencing.



Alternate QC Methods

See Appendix for representative traces:

- Agilent TapeStation Traces on page 107
- LabChip Traces on page 108

See Appendix for Post Library Construction Quantification on page 106

Step 7:

cDNA Amplification

7.0 Get Started 79

7.1 cDNA Amplification 79

7.2 cDNA Cleanup – SPRIselect 80

7.3 cDNA QC & Quantification 81

7.0 Get Started

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature					
☐	●	cDNA Primers	2000089	Vortex, centrifuge briefly.	−20°C
☐		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
☐		Agilent Bioanalyzer High Sensitivity Kit *(if used for QC and quantification)*	-	Manufacturer's recommendations.	-
☐		Agilent TapeStation ScreenTape and Reagents *(if used for QC and quantification)*	-	Manufacturer's recommendations.	-
☐		Qubit dsDNA HS Assay Kit *(if used for QC and quantification)*	-	Manufacturer's recommendations.	-
Place on ice					
☐	○	Amp Mix	2000047	Vortex, centrifuge briefly.	−20°C
Obtain					
☐		Qiagen Buffer EB	-	Manufacturer's recommendations.	-
☐		10x Magnetic Separator	230003	-	Ambient
☐		Prepare 80% Ethanol - *Prepare 15 ml for 8 reactions.*		-	-

7.1 cDNA Amplification

a. Prepare cDNA Amplification Mix on ice. Vortex and centrifuge briefly.

	cDNA Amplification Reaction Mix Add reagents in the order listed	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○	Amp Mix	2000047	50	220	440
●	cDNA Primers	2000089	15	66	132
	Total	-	**65**	**286**	**572**

b. Transfer **35 µl** pre-amplified sample from step 5.3 to a new tube strip. Add **65 µl** cDNA Amplification Reaction Mix to the sample.

c. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
105°C	100 µl	~30-45 min
Step	**Temperature**	**Time**
1	98°C	00:03:00
2	98°C	00:00:15
3	63°C	00:00:20
4	72°C	00:01:00
5	Go to Step 2, see table below for total # of cycles	
6	72°C	00:01:00
7	4°C	Hold

The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. The number of cDNA cycles should also be reduced if large numbers of nuclei are sampled.

Recommended starting point for cycle number optimization.

Targeted Nuclei Recovery	Total Cycles
≤2,000	9
2,001–6,000	7
≥6,001	6

e. Store at **4°C** for up to **72 h** or **−20°C** for **≤1 week**, or proceed to the next step.

7.2 cDNA Cleanup – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect reagent (**0.6X**) to each sample and pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly and place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

j. Remove from the magnet. Add **40.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place the tube strip on the magnet•**High** until the solution clears.

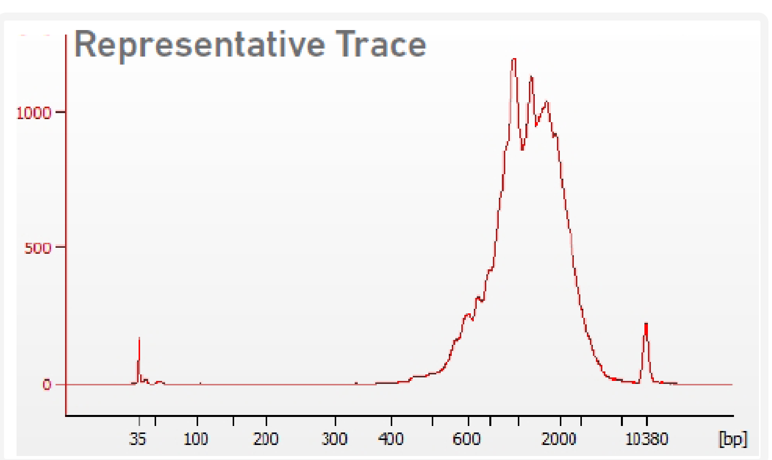
m. Transfer **40 µl** sample to a new tube strip.

n. Store at **4°C** for up to **72 h** or at **−20°C** for up to **4 weeks**, or proceed to the next step.

7.3 cDNA QC & Quantification

a. Run 1 µl undiluted sample on an Agilent Bioanalyzer High Sensitivity chip.

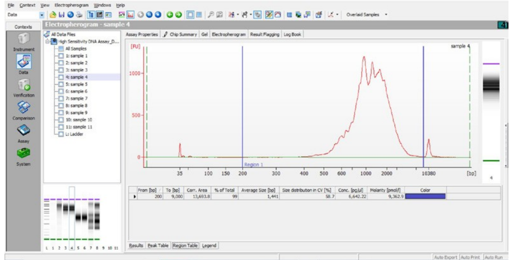
Depending on specific sample, it may also be diluted 1:2. Lower molecular weight product (35 – 150 bp) may be present on the traces. This is normal and does not affect sequencing or application performance.



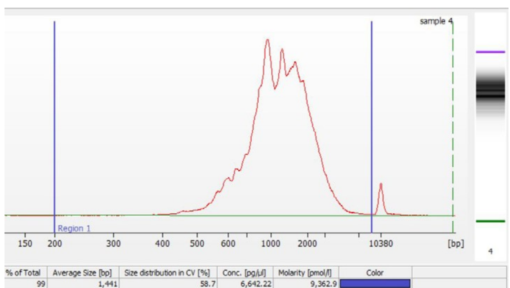
EXAMPLE CALCULATION

i. Select Region

Under the "Electropherogram" view choose the "Region Table". Manually select the region of ~200 – ~9000 bp



ii. Note Concentration [pg/µl]



iii. Calculate

Multiply the cDNA concentration [pg/µl] reported via the Agilent 2100 Expert Software by the elution volume (40 µl) of the Post cDNA Amplification Reaction Clean Up sample (taking any dilution factors into account) and then divide by 1000 to obtain the total cDNA yield in ng.

Example Calculation of cDNA Total Yield

Concentration: 6642.22 pg/µl
Elution Volume: 40
Dilution Factor: 1
Total cDNA Yield

= Conc'n (pg/µl) x Elution Volume (µl) x Dilution Factor
1000 (pg/ng)

= 6642.22 (pg/µl) x 40 (µl) x 1 = 265.69 ng
1000 (pg/ng)

Carry forward ONLY 25% of total cDNA yield into 3' Gene Expression Library Construction (step 8)

= 0.25 x Total cDNA yield

= 0.25 x 265.69= 66.42 ng

Refer to step 8.5 for appropriate number of Sample Index PCR cycles based on carry forward cDNA yield/input cDNA.

Alternate QC Methods

See Appendix for representative traces:

- Agilent TapeStation Traces on page 107
- LabChip Traces on page 108

Agilent Bioanalyzer, Agilent TapeStation, or LabChip are the recommended methods for accurate quantification.

Step 8:

Gene Expression Library Construction

8.0 Get Started 84

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8.1 Fragmentation, End Repair & A-tailing 86

8.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect 87

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8.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect 91

8.7 Post Library Construction QC 92

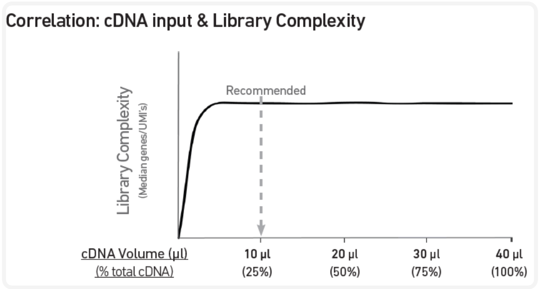
8.0 Get Started

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature					
☐	●	Fragmentation Buffer	2000091/ 2001316	Vortex, verify no precipitate, centrifuge briefly.	−20°C
☐	●	Ligation Mix	2001109/ 2001317	Thaw, vortex, verify no precipitate, centrifuge.	−20°C
☐		Dual Index Plate TT Set A	3000431	-	−20°C
☐		Beckman Coulter SPRIselect Reagent	-	Manufacturer's recommendations.	-
☐		Agilent TapeStation Screen Tape and Reagents *(if used for QC)*		Manufacturer's recommendations.	
☐		Agilent Bioanalyzer High Sensitivity kit *(if used for QC)*	-	Manufacturer's recommendations.	-
☐		DNA High Sensitivity Reagent Kit *(if LabChip used for QC)*	-	Manufacturer's recommendations.	-
Place on Ice					
☐	●	Fragmentation Enzyme	2000090/ 2000104/ 2001315	Centrifuge briefly.	−20°C
☐	●	DNA Ligase	220110/ 220131/2001318	Centrifuge briefly.	−20°C
☐	○	Library Amp Mix or Amp Mix	2000531 or 2000047/2001319	Centrifuge briefly.	−20°C
☐		KAPA Library Quantification Kit for Illumina Platforms	-	Manufacturer's recommendations.	-
Obtain					
☐		Qiagen Buffer EB	-	-	Ambient
☐		10x Magnetic Separator	230003	See Tips & Best Practices.	Ambient
☐		Prepare 80% Ethanol *Prepare 20 ml for 8 reactions*	-	Prepare fresh.	Ambient

Step Overview (Step 8.1d)

Correlation between input & library complexity

A Single Cell Gene Expression library is generated using a fixed proportion (10 µl, 25%) of the total cDNA (40 µl) obtained at step 7.2n. The complexity of this library will be comparable to one generated using a higher proportion (>25%) of the cDNA. The remaining proportion (30 µl, 75%) of the cDNA may be stored at **4°C** for up to **72 h** or at **−20°C** for longer-term storage (up to **4 weeks**).



Note that irrespective of the total cDNA yield (ng), which may vary based on cell type, targeted nuclei recovery etc., this protocol has been optimized for a broad range of input mass (ng), as shown in the example below. The total number of SI PCR cycles (step 8.5d) should be optimized based on carrying forward a fixed proportion (10 µl, 25%) of the total cDNA yield calculated during Post cDNA Amplification QC & Quantification (step 7.3).

Example: Library Construction Input Mass & SI PCR Cycles

Cell Type	Targeted Nuclei Recovery	Total cDNA Yield (ng)	cDNA Input into Fragmentation		SI PCR Cycle Number
			Volume (µl)	Mass (ng)	
High RNA Content	Low	150 ng	10 µl	37.5 ng	14
	High	400 ng	10 µl	100 ng	13
Low RNA Content	Low	1 ng	10 µl	0.25 ng	16
	High	100 ng	10 µl	25 ng	14

The term "cell" as used here applies to nuclei too.

8.1 Fragmentation, End Repair & A-tailing

a. Prepare a thermal cycler with the following incubation protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
65°C	50 µl	~35 min
Step	**Temperature**	**Time**
1. Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
2. Fragmentation	32°C	00:05:00
3. End Repair & A-tailing	65°C	00:30:00
4. Hold	4°C	Hold

b. Vortex Fragmentation Buffer. Verify there is no precipitate.

c. Prepare Fragmentation Mix on ice. Pipette mix and centrifuge briefly.

Fragmentation Mix Add reagents in the order listed	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer EB	-	25	110	220
Fragmentation Buffer	2000091/2001316	5	22	44
Fragmentation Enzyme	2000090/2000104/2001315	10	44	88
Total	-	**40**	**176**	**352**

d. Transfer **ONLY 10 µl** purified cDNA sample from cDNA Cleanup (step 7.2n) to a tube strip.

Note that only **10 µl** (25%) cDNA sample is sufficient for generating 3′ Gene Expression library. The remaining **30 µl** (75%) cDNA sample can be stored at **4°C** for up to **72 h** or at **−20°C** for up to **4 weeks** for generating additional Gene Expression libraries.

e. Add **40 µl** Fragmentation Mix to each sample.

f. Pipette mix 15x (pipette set to 35 µl) on ice. Centrifuge briefly.

g. Transfer into the pre-cooled thermal cycler (**4°C**) and press "SKIP" to initiate the protocol.

8.2 Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **30 µl** SPRIselect (**0.6X**) reagent to each sample. Pipette mix 15x (pipette set to 75 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **75 µl** supernatant to a new tube strip.

e. Vortex to resuspend SPRIselect reagent. Add **10 µl** SPRIselect reagent (**0.8X**) to each sample. Pipette mix 15x (pipette set to 80 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove **80 µl** supernatant. DO NOT discard any beads.

i. Add **125 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low** until the solution clears. Remove remaining ethanol. DO NOT dry.

m. Remove from the magnet and immediately add **50.5 µl** Buffer EB to each sample. Pipette mix 15x.

n. Incubate **2 min** at **room temperature**.

o. Place on the magnet•**High** until the solution clears.

p. Transfer **50 µl** sample to a new tube strip.

8.3 Adaptor Ligation

a. Prepare Adaptor Ligation Mix. Pipette mix and centrifuge briefly.

	Adaptor Ligation Mix Add reagents in the order listed	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
●	Ligation Mix	2001109/2001317	40	176	352
●	DNA Ligase	220110/ 220131/2001318	10	44	88
	Total	-	**50**	**220**	**440**

b. Add **50 µl** Adaptor Ligation Mix to **50 µl** sample. Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

c. Incubate in a thermal cycler with the following protocol.

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

8.4 Post Ligation Cleanup – SPRIselect

a. Vortex to resuspend SPRIselect Reagent. Add **80 µl** SPRIselect Reagent (**0.8X**) to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears.

d. Remove the supernatant.

e. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

f. Remove the ethanol.

g. **Repeat** steps e and f for a total of 2 washes.

h. Centrifuge briefly. Place on the magnet•**Low**.

i. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

j. Remove from the magnet. Add **30.5 µl** Buffer EB. Pipette mix 15x.

k. Incubate **2 min** at **room temperature**.

l. Place on the magnet•**Low** until the solution clears.

m. Transfer **30 µl** sample to a new tube strip.

8.5 Sample Index PCR

a. Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Record the 10x sample index name (PN-3000431 Dual Index Plate TT Set A well ID) used.

b. Add **50 µl** Library Amp Mix (PN-2000531) or Amp Mix (PN2000047/2000103) to **30 µl** sample.

c. Add **20 µl** of an individual Dual Index TT Set A to each well and record the well ID used. Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.

d. Incubate in a thermal cycler with the following protocol.

Lid Temperature	Reaction Volume	Run Time hh:mm:ss
105°C	100 µl	~25-40 min
Step	**Temperature**	**Time**
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

The total cycles should be optimized based on 25% carry forward cDNA yield/input calculated during cDNA QC & Quantification on page 81

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
>1500 ng	5

e. Store at **4°C** for up to **72 h** or proceed to the next step.

8.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect

a. Vortex to resuspend the SPRIselect reagent. Add **60 µl** SPRIselect Reagent (**0.6X**) to each sample. Pipette mix 15x (pipette set to 150 µl).

b. Incubate **5 min** at **room temperature**.

c. Place on the magnet•**High** until the solution clears. DO NOT discard supernatant.

d. Transfer **150 µl** supernatant to a new tube strip.

e. Vortex to resuspend the SPRIselect reagent. Add **20 µl** SPRIselect Reagent (**0.8X**) to each sample (supernatant). Pipette mix 15x (pipette set to 150 µl).

f. Incubate **5 min** at **room temperature**.

g. Place the magnet•**High** until the solution clears.

h. Remove **165 µl** supernatant. DO NOT discard any beads.

i. With the tube still in the magnet, add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. **Repeat** steps i and j for a total of 2 washes.

l. Centrifuge briefly. Place on the magnet•**Low**. Remove remaining ethanol.

   Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

m. Remove from the magnet. Add **35.5 µl** Buffer EB. Pipette mix 15x.

n. Incubate **2 min** at **room temperature**.

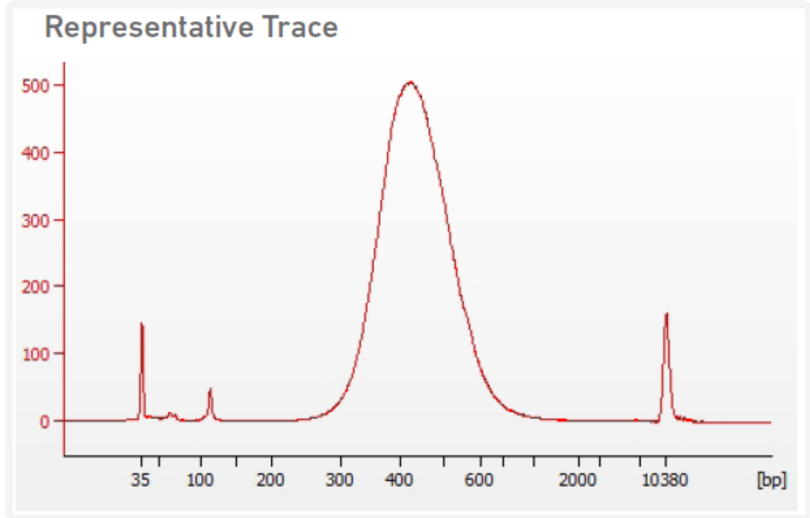
o. Place on the magnet•**Low** until the solution clears.

p. Transfer **35 µl** to a new tube strip.

q. Store at **4°C** for up to **72 h** or at **−20°C** for **long-term storage**.

8.7 Post Library Construction QC

Run 1 µl sample at 1:10 dilution on an Agilent Bioanalyzer High Sensitivity chip.



Determine the average fragment size from the Bioanalyzer trace. This will be used as the insert size for library quantification.

Alternate QC Methods

See Appendix for representative traces:

- Agilent TapeStation Traces on page 107
- LabChip Traces on page 108

See Appendix for Post Library Construction Quantification on page 106

Step 9:

Sequencing

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Sample Indices 95

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Gene Expression Library Sequencing Depth & Run Parameters 96

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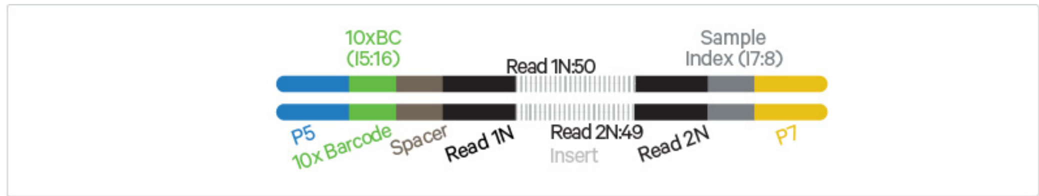
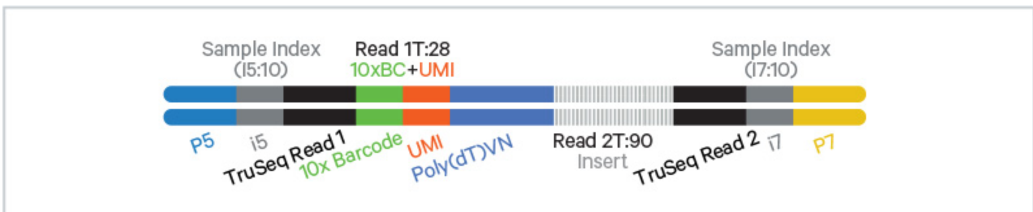
Library Pooling 97

Sequencing Libraries

Chromium Single Cell Multiome ATAC libraries comprise double stranded DNA with standard Illumina® paired-end constructs which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder that includes paired-end Read 1N and Read 2N used for sequencing the DNA insert, 8 bp sample index in the i7 read, and 24 bp in the i5 read to cover the 16 bp 10x Barcode and 8 bp Spacer.



Chromium Single Cell Multiome Gene Expression libraries comprise cDNA insert with standard Illumina® paired-end constructs which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder. TruSeq Read 1 is used to sequence 16 bp 10x Barcodes and 12 bp UMI, while 10 bp i5 and i7 sample index sequences are the sample index reads. TruSeq Read 2 is used to sequence the insert.



Sequencer Compatibility

10x Genomics libraries contain P5 and P7 adaptors, which can be used for Illumina® sequencing.

The compatibility of the listed sequencers has been verified by 10x Genomics. For a list of additional sequencers, consult the Sequencer Compatibility page on the 10x Genomics support site. Some variation in assay performance is expected based on sequencer choice.

- MiSeq™
- NextSeq™ 500/550/2000
- HiSeq 2500™ (Rapid Run)
- HiSeq™ 3000/4000

- NovaSeq™ 6000
- NovaSeq™ X Series

Sample Indices

Each sample index in the Dual Index Kit TT Set A (PN-1000215) is a mix of one unique i7 and one unique i5 sample index. Each i7 sample index in the Single Index Kit N Set A (PN-1000212) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. Single Index Plate_ Set_well ID) is needed in the sample sheet used for generating FASTQs with Cell Ranger. Samples utilizing the same sample index should not be pooled together or run on the same flow cell lane, as this would not enable correct sample demultiplexing.

ATAC Library Sequencing Depth & Run Parameters

These sequencing parameters are specific for Chromium Single Cell Multiome ATAC libraries and are different from the recommendations for standalone ATAC libraries. Ensure that these specific recommendations are provided to the sequencing service provider.

Sequencing Depth	25,000 read pairs per nucleus (25,000 reads for Read 1N; 25,000 reads for Read 2N)
Sequencing Type	Paired-end, dual indexing
Sequencing Read	
Read 1N	50 cycles
i7 Index	8 cycles
i5 Index	24 cycles*
Read 2N	49 cycles

***Custom sequencing recipe:**

Sequencers that do not support 24 nt read in i5 (e.g. Nextseq™ 500/550) require a custom recipe that includes 8 dark cycles and 16 nt cycles on i5. After installation of custom sequencing recipe, input 16 cycles for i5 read.

It is **imperative to use a custom recipe** for these sequencers. Entering 16 cycles for i5 read without the use of the custom recipe will cause the sequencing run to proceed but the data will be unusable.

Custom recipe is NOT required for NextSeq™ 1000/2000.

Contact Support@10xgenomics.com for any additional questions.

ATAC Library Loading

Once quantified and normalized, libraries should be denatured and diluted as recommended for Illumina® sequencing platforms. Refer to Illumina® documentation for denaturing and diluting libraries. Refer to the 10x Genomics Support website for more information.

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq™	10	1
NextSeq™ 500/550	1.5	1
NextSeq™ 1000/2000	650	1
HiSeq™ 2500 (RR)	10	1
HiSeq™ 4000	180	1
NovaSeq™ 6000 Standard & Xp workflow	150	1
NovaSeq™ X Series	150-200	1

Gene Expression Library Sequencing Depth & Run Parameters

Sequencing Depth	20,000 read pairs per nucleus
Sequencing Type	Paired-end, dual indexing
Sequencing Read	Recommended Cycles
Read 1	28 cycles
i7 Index	10 cycles
i5 Index	10 cycles
Read 2	90 cycles

Gene Expression Library Loading

Once quantified and normalized, libraries should be denatured and diluted as recommended for Illumina® sequencing platforms. Refer to Illumina® documentation for denaturing and diluting libraries. Refer to the 10x Genomics Support website for more information.

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq™	11	1
NextSeq™ 500/550	1.8	1
NextSeq™ 1000/2000	650	1
HiSeq™ 2500 (RR)	11	1
HiSeq™ 4000	240	1
NovaSeq™ 6000 Standard & Xp workflow	150	1
NovaSeq™ X Series	150-200	1

Library Pooling

Single Cell Multiome ATAC libraries may be pooled with other ATAC libraries only when using forward strand Illumina® workflow. Single Cell Multiome Gene Expression libraries may be pooled for sequencing with other libraries, taking into account the differences in cell number and per-cell read depth requirements between each library. Samples utilizing the same sample index should not be pooled together, or run on the same flow cell lane, as this would not enable correct sample demultiplexing. Refer to the 10x Genomics Support website for more information.

Refer to Batch Effect with Different Chemistry Versions on page 111 for information regarding batch effects when analyzing libraries generated using different chemistry versions.

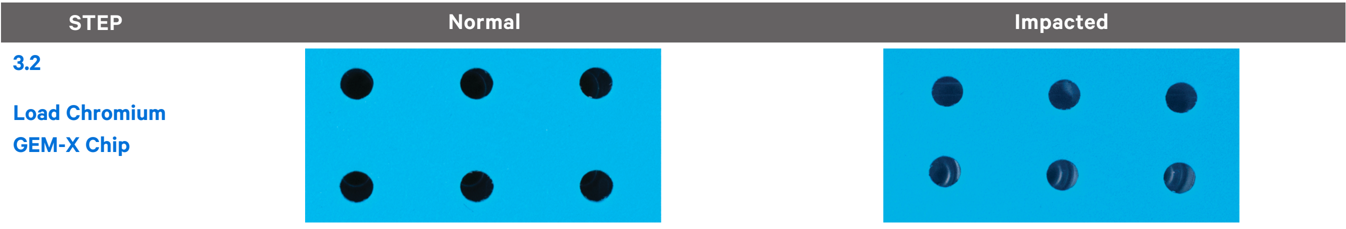
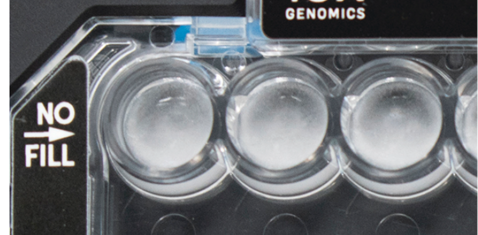
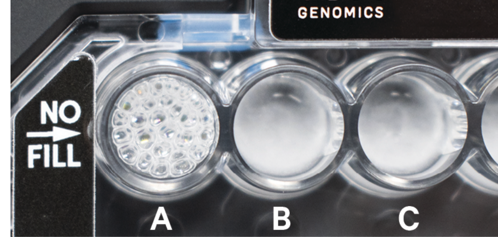
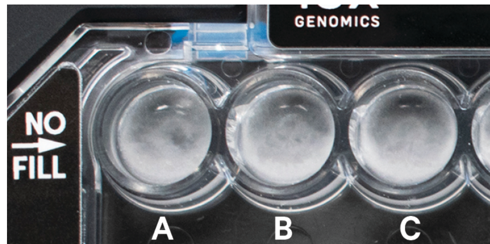
Troubleshooting

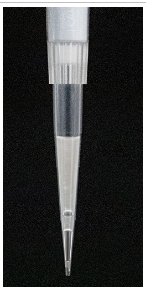
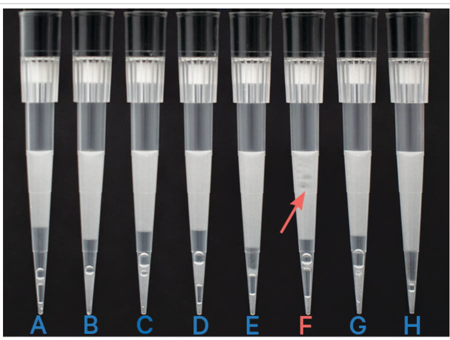
GEM Generation & Barcoding 99

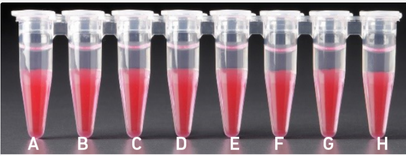
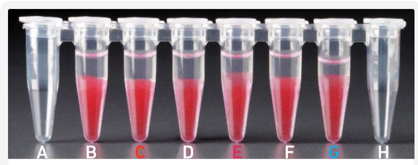
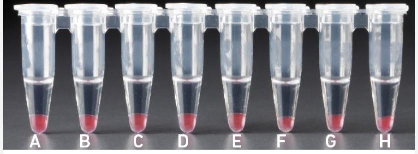
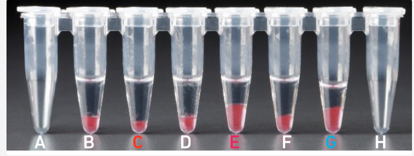
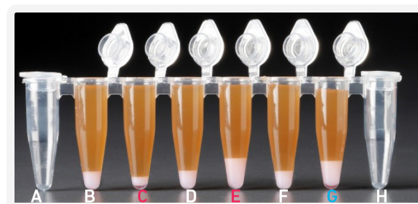
Post library Construction QC 103

Chromium X Series Errors 104

GEM Generation & Barcoding

STEP	Normal	Impacted
3.2 **Load Chromium GEM-X Chip**	 Gasket holes are aligned with the sample and gel bead wells.	Gasket holes are misaligned with the gel bead wells. Open and close the chip holder slowly once. *One of the rows of Chromium X/iX gasket will not align with the GEM-X chip. This is normal and will not impact the assay.*
3.4 d **After chip is removed from the instrument and the wells are exposed.**	 All recovery wells are similar in volume and opacity.	 Recovery well A indicates a wetting failure. Not all wetting failures may present themselves with excess bubbles (foam).  Recovery wells A-C show normal emulsions. Not all normal emulsions may have small (in size and number) bubbles in the recovery wells. **Inspecting emulsions in pipette tips is recommended for diagnosing emulsion failures.**

STEP	Normal	Impacted
3.4 e **Transfer GEMs from chip**		 Wetting failure Normal emulsion
	All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	Pipette tip A shows wetting failure. Pipette tips B-C show uniform emulsions & slightly low volumes. (~95 µl) Most wetting failures will not impact emulsion volumes of other sample run on the chip. Occasionally, wetting failures may impact the emulsion volumes recovered from other lanes. This is expected and does not indicate an emulsion failure if the samples are uniform in volume and opacity (B-C).
	 Pipette tip shows recovery of slightly lower emulsion volume (90-95 µl). This is not a clog, but a pipetting error during chip loading or emulsion recovery. It is acceptable to proceed to the next steps in this case but the cell recovery may be slightly lower. To prevent this, use a calibrated pipette and follow the loading and recovery instructions closely.	
		In pipette tips A-E & G-H, a portion of the emulsion displays separate layers of oil, which indicates clog. Pipette tip F indicates a clog (separate oil layers) and wetting failure (non-uniform emulsion).
	Emulsion failures are not expected to be observed across the entire chip. The above images are for illustrative purposes to show a range in emulsion failures. A clog is not expected to impact other lanes on the chip.	

STEP	Normal	Impacted
4.1 a **After transfer of the GEMs + Recovery Agent**	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/ Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).
4.1 b **After aspiration of Recovery Agent/ Partitioning Oil**	 All liquid volumes are similar in the aqueous sample volume (clear) and residual Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). There is also a greater residual volume of Recovery Agent/ Partitioning Oil (pink). Tube C and E indicate a wetting failure has occurred. There is an abnormal residual volume of Recovery Agent/Partitioning Oil (pink).
4.1 d **After addition of Dynabeads Cleanup Mix**	 All liquid volumes are similar after addition of the Dynabeads Cleanup Mix.	 Tube G indicates a reagent clog has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white). Tube C and E indicate a wetting failure has occurred. There is an abnormal ratio of Dynabeads Cleanup Mix (brown) to Recovery Agent/Partitioning Oil (appears white).

After GEM generation, it is strongly recommended to always take a picture of:

- Emulsion(s) in the chip (horizontal and at 45 degrees)
- Emulsion(s) in the pipette tip/tube
- The chip after emulsions have been recovered

If a channel clog or wetting failure is documented in one or more samples in a set:

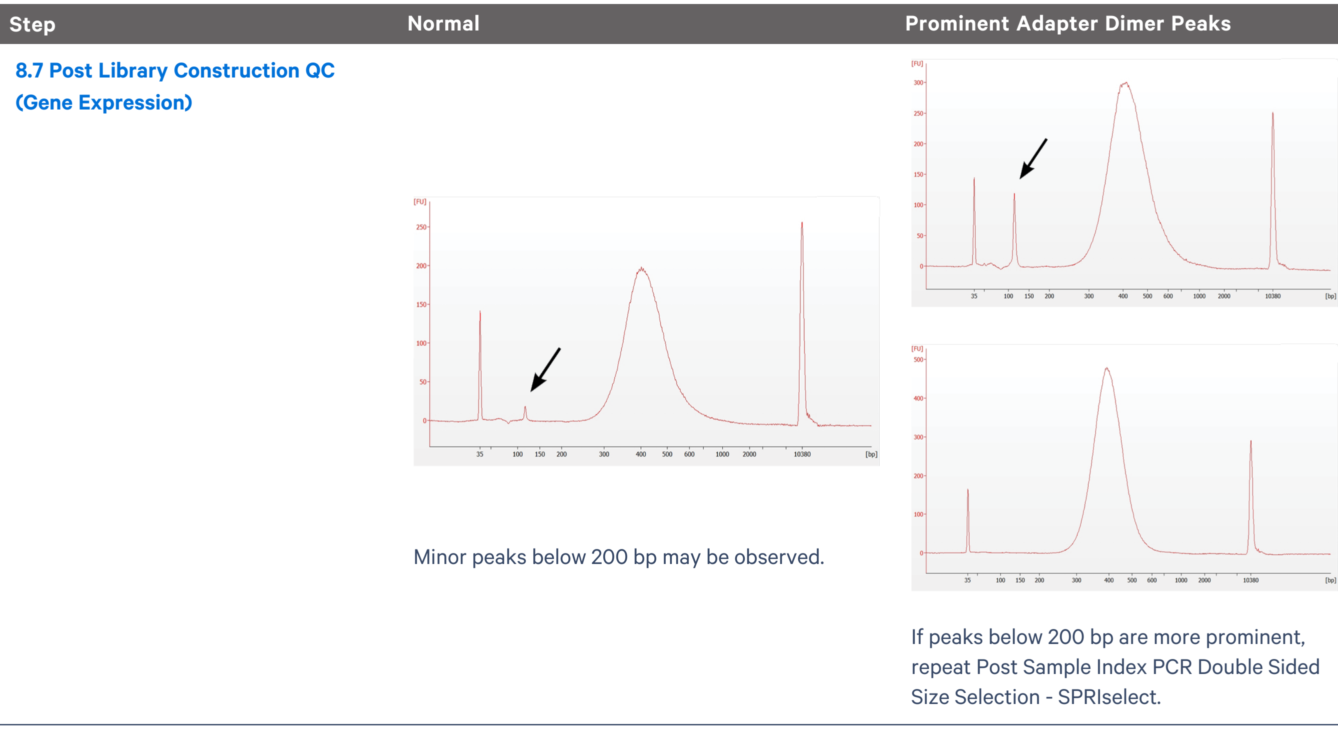
- Data can be derived from the remaining samples in the set by immediately proceeding with GEM-RT

  OR

- Data from all the samples in a set can be derived by immediately restarting GEM generation. If restarting GEM generation for one set of samples, ensure that a thermal cycler is available for the second set as GEM-RT incubation should start within 5 min of GEM recovery.

Contact support@10xgenomics.com for further assistance. Replacement reagents and chips may be provided for properly documented clogs or wetting failures if they are associated with runs of unexpired reagents and chips, and are reported within 30 days of the expiration date.

Post library Construction QC



Step	Normal	Prominent Adapter Dimer Peaks
8.7 Post Library Construction QC (Gene Expression)	Minor peaks below 200 bp may be observed.	If peaks below 200 bp are more prominent, repeat Post Sample Index PCR Double Sided Size Selection - SPRIselect.

Chromium X Series Errors

The Chromium X touchscreen will guide the user through recoverable errors. If the error continues, or if the instrument has seen critical or intermediate errors, email support@10xgenomics.com with the displayed error code. Support will request a troubleshooting package. Upload pertinent logs to 10x Genomics by navigating to the Logs menu option on screen.

There are two types of errors:

Critical Errors — When the instrument has seen a critical error, the run will immediately abort. Do not proceed with any further runs. Contact support@10xgenomics. com with the error code.

a. System Error
b. Pressure Error
c. Chip Error
d. Run Error
e. Temperature Error
f. Software Error

User Recoverable Errors — Follow error handling instructions through the touchscreen and continue the run.

a. Gasket Error
b. Tray Error
c. Chip Error
d. Unsupported Chip Error
e. Update Error

Consult the Chromium X Series (X/iX) User Guide (CG000396) for additional information and follow the Chromium X touchscreen prompts for execution.

Appendix

Post Library Construction Quantification 106

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Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina Platforms.

b. Dilute **2 µl** sample with deionized water to appropriate dilutions that fall within the linear detection range of the KAPA Library Quantification Kit for Illumina Platforms. (For more accurate quantification, make the dilution(s) in duplicate).

c. Make enough Quantification Master Mix for the DNA dilutions per sample and the DNA Standards (plus 10% excess) using the guidance for 1 reaction volume below.

Quantification Master Mix	1X (µl)
SYBR Fast Master Mix + Primer	12
Water	4
Total	16

d. Dispense **16 µl** Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.

e. Add **4 µl** sample dilutions and **4 µl** DNA Standards to appropriate wells. Centrifuge briefly.

f. Incubate in a thermal cycler with the following protocol.

Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C Read Signal	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration based on average insert size derived from the Bioanalyzer/TapeStation trace.

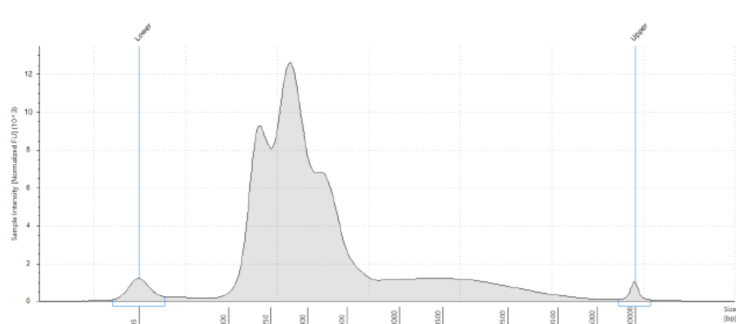
Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used.

Protocol Step 6.3 – Post Library Construction QC (ATAC Library)

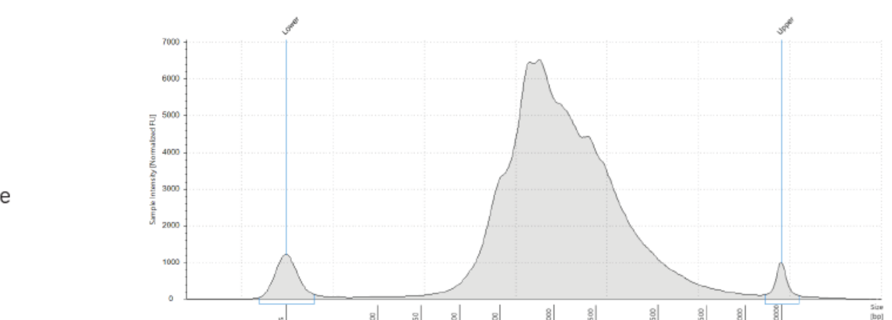
Run 2 µl sample at 1:2 dilution

Run 2 µl sample

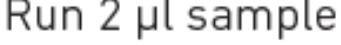


Protocol Step 7.3 – cDNA QC & Quantification

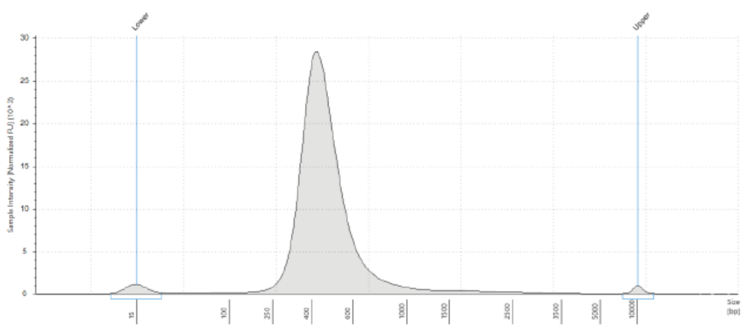
Run 2 µl sample



Protocol Step 8.7 – Post Library Construction QC (Gene Expression Library)



Run 2 µl sample



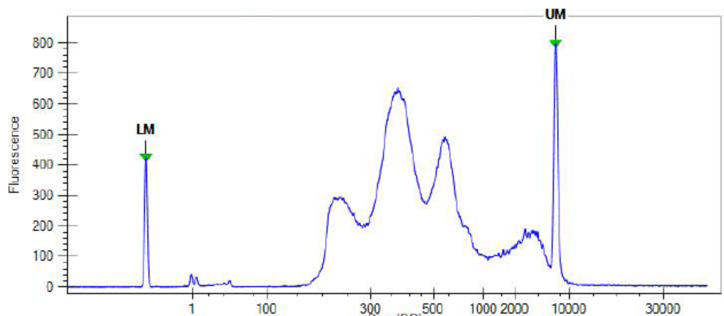
All traces are representative

LabChip Traces

DNA High Sensitivity Reagent Kit was used.

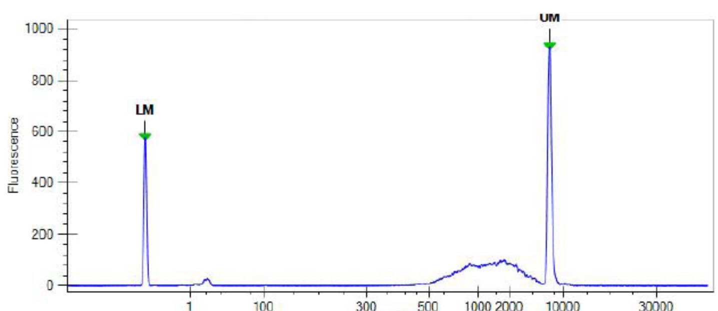
Protocol Step 6.3 – Post Library Construction QC (ATAC Library)

Run 10 µl undiluted sample



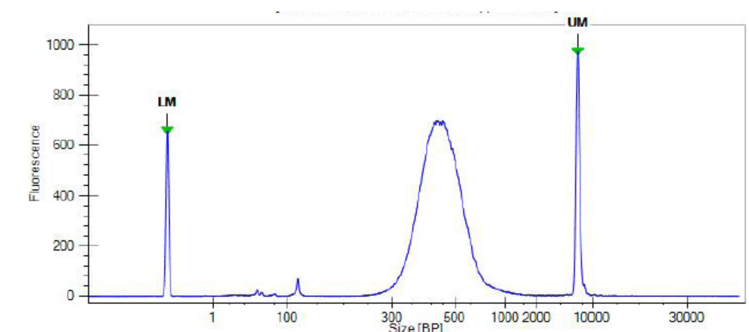
Protocol Step 7.3 – cDNA QC & Quantification

Run 10 µl undiluted sample



Protocol Step 8.7 – Post Library Construction QC (Gene Expression Library)

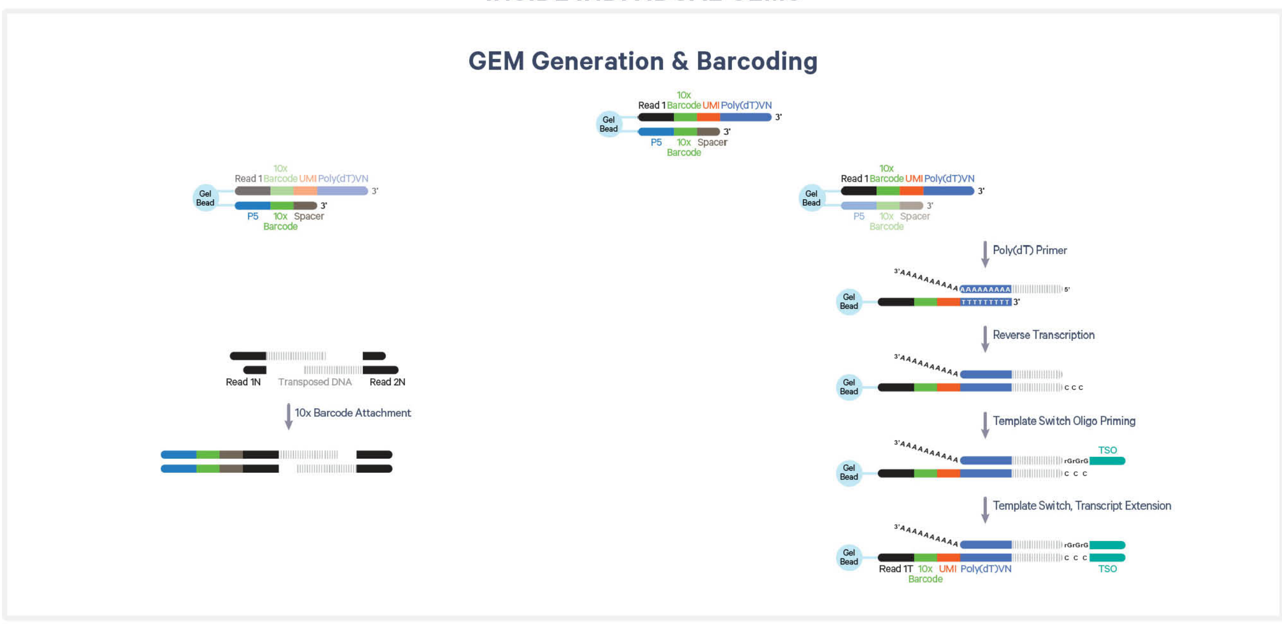
Run 10 µl undiluted sample



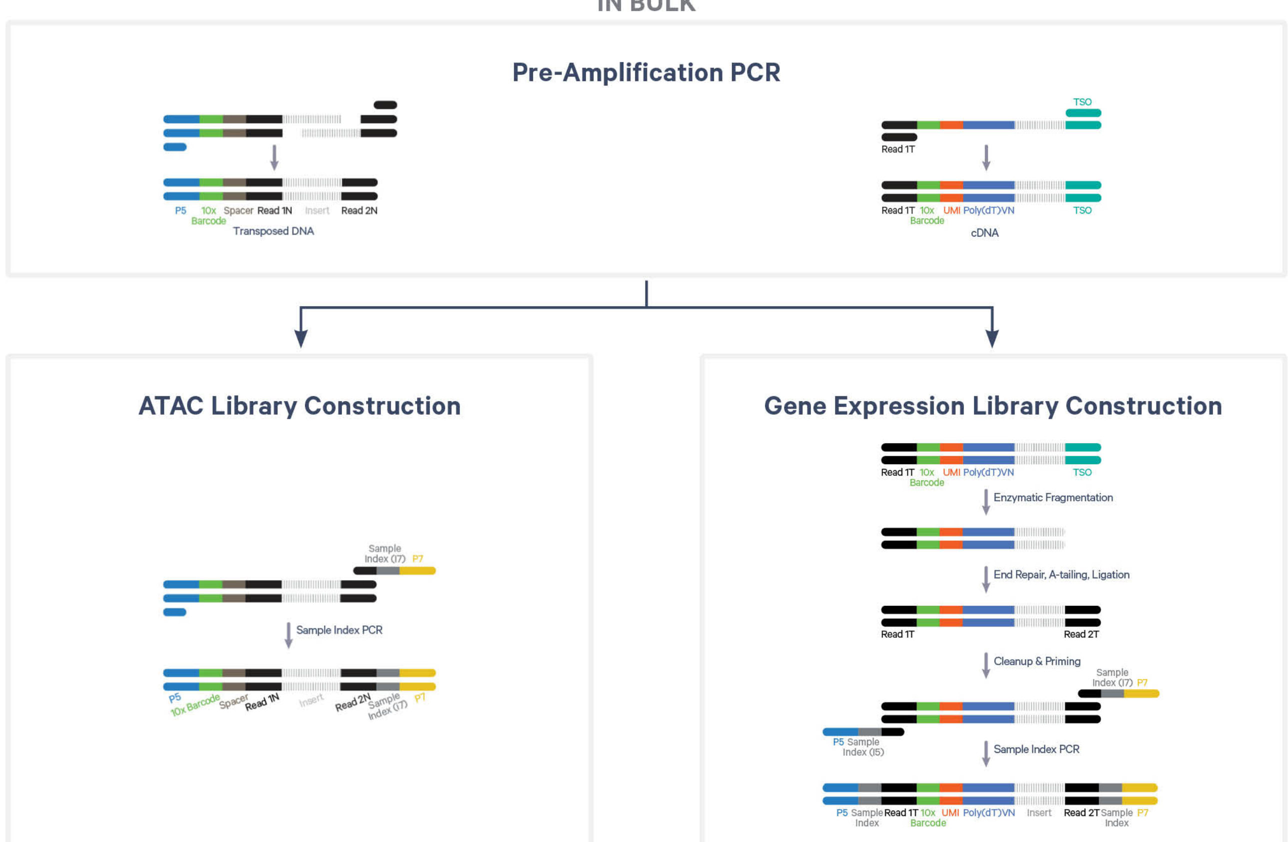
All traces are representative.

Assay Scheme Overview

INSIDE INDIVIDUAL GEMS

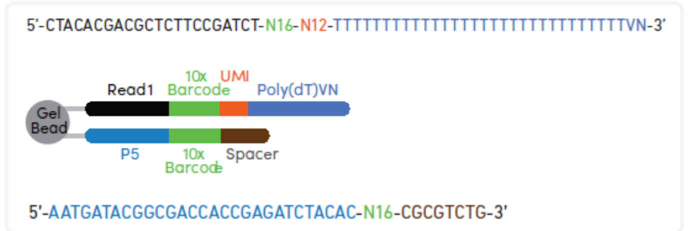


IN BULK

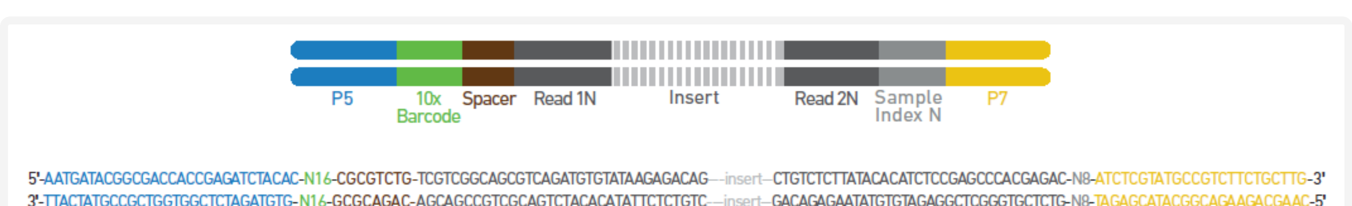


Sequences

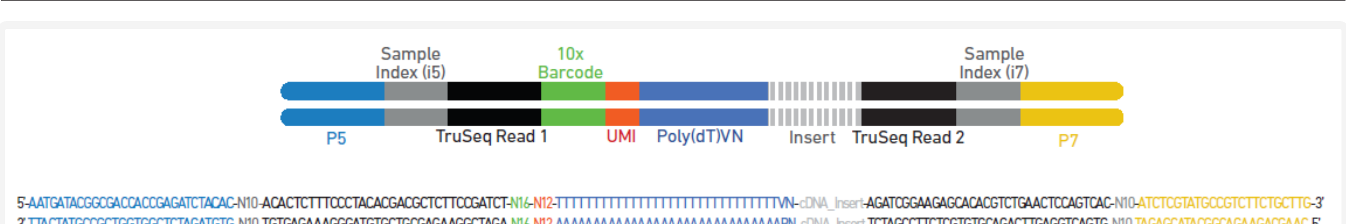
Single Cell Multiome Gel Beads A (PN-2000261)



Chromium Single Cell Multiome ATAC Library

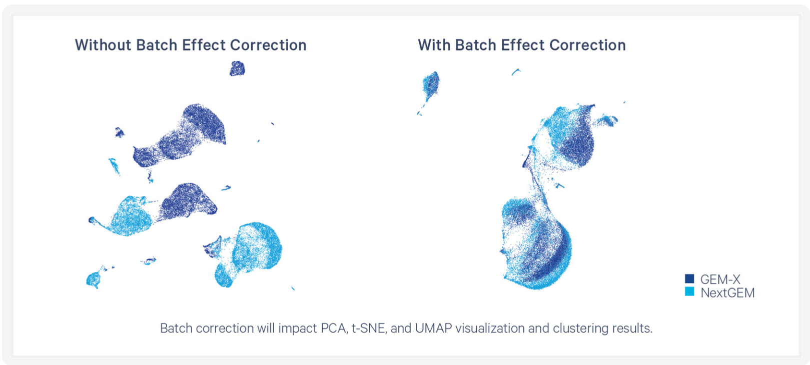


Chromium Single Cell Multiome Gene Expression Library

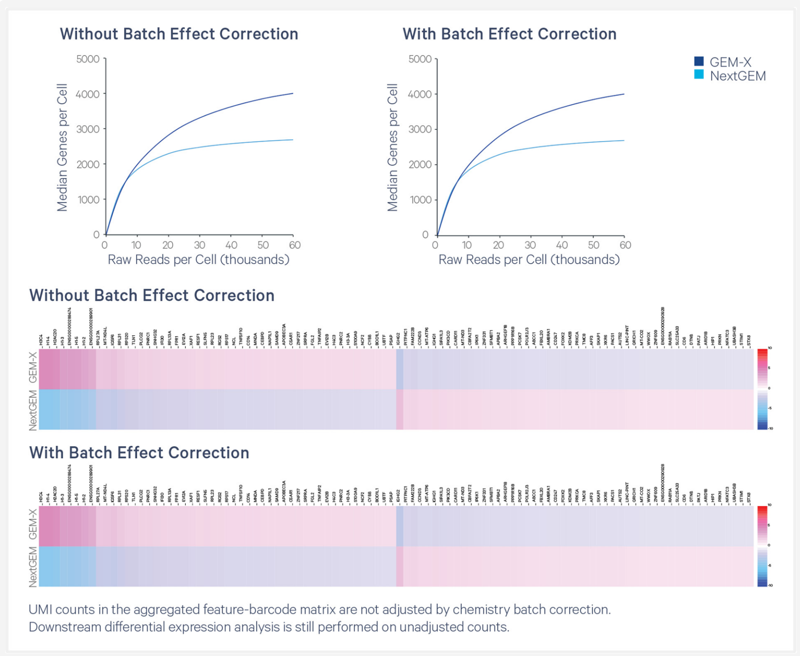


Batch Effect with Different Chemistry Versions

Careful planning is needed to manage potential batch effects when analyzing data from different chemistry versions. One way to do this is by using cellranger aggr, which has a built-in chemistry batch effect correction option to combine data.



Batch correction will impact PCA, t-SNE, and UMAP visualization and clustering results.



UMI counts in the aggregated feature-barcode matrix are not adjusted by chemistry batch correction. Downstream differential expression analysis is still performed on unadjusted counts.

The term "cell" as used here applies to nuclei too.

The degree of correction may vary based on sample type and experimental conditions.