CG000338 Rev G

USER GUIDE

Chromium Next GEM Single Cell Multiome ATAC + Gene Expression

FOR USE WITH

Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Bundle, 16 rxns PN-1000283, includes:

- Chromium Next GEM Single Cell Multiome ATAC Kit A, 16 rxns PN-1000280
- Chromium Next GEM Single Cell Multiome Reagent Kit A, 16 rxns PN-1000282
- Library Construction Kit, 16 rxns PN-1000190

Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Bundle, 4 rxns PN-1000285, includes:

- Chromium Next GEM Single Cell Multiome ATAC Kit A, 4 rxns PN-1000281
- Chromium Next GEM Single Cell Multiome Reagent Kit A, 4 rxns PN-1000284
- Library Construction Kit B, 4 rxns PN-1000279

Chromium Next GEM Chip J Single Cell Kit, 48 rxns PN-1000234

Chromium Next GEM Chip J Single Cell Kit, 16 rxns PN-1000230

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

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

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

Notices

Document Number

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Legal Notices

Instrument & Licensed Software Updates Warranties

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

Support

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Document Revision Summary

Document Number	CG000338
Title	Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide
Revision	Rev F to Rev G
Revision Date	September 2024

Specific Changes:

- **Updated thermal cycler recommendations (page 13)**
- **Updated to include 10x Magnetic Separator B (page 13, 25, 39, 44) and Chromium X Series Accessory Kit (page13)**
- **Updated glycerol loading volume for row labeled 3, step 2.2a (page 34)**
- **Updated to include Novaseq™6000 guidance (pages 70-71)**
- **Updated to remove QuBit for QC**

General Changes:

- **Updated for general minor consistency of language and terms throughout.**

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Chromium Next GEM Single Cell Multiome Reagent Kit A, 16 rxns PN-1000282

Chromium Next GEM Single Cell Multiome GEM Kit A, 16 rxns PN-1000232 (store at –20°C)

Chromium
Next GEM Single Cell Multiome GEM Kit A
Store at -20°C

	#	PN
Barcoding Reagent Mix	1	2000267
Barcoding Enzyme Mix	1	2000266
Template Switch Oligo	1	3000228
Reducing Agent B	1	2000087
Cleanup Buffer	2	2000088
Quenching Agent	1	2000269

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Chromium Next GEM Single Cell Multiome Amp Kit A, 16 rxns PN-1000233 (store at –20°C)

Chromium
Next GEM Single Cell Multiome Amp Kit A
Store at -20°C

	#	PN
Pre-Amp Mix	1	2000270
Pre-Amp Primers	1	2000271
Amp Mix	2	2000047
SI-PCR Primer B	1	2000128
cDNA Primers	1	2000089

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell Multiome Gel Bead Kit A, 16 rxns PN-1000231 (store at –80°C)

Chromium
Next GEM Single Cell Multiome Gel Beads A
Store at -80°C

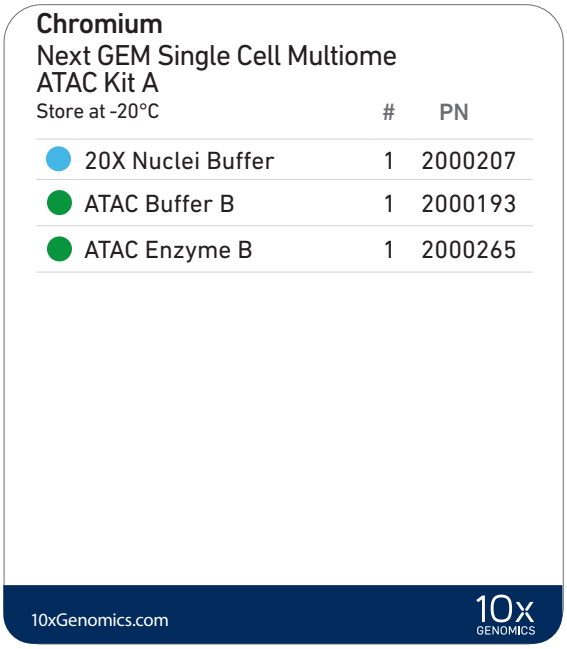
	#	PN
Single Cell Multiome Gel Beads A	2	2000261

10xGenomics.com 10x GENOMICS

Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	2	2000048

Chromium Next GEM Single Cell Multiome ATAC Kit A, 16 rxns PN-1000280 (store at –20°C)



Chromium
Next GEM Single Cell Multiome ATAC Kit A
Store at -20°C

	#	PN
20X Nuclei Buffer	1	2000207
ATAC Buffer B	1	2000193
ATAC Enzyme B	1	2000265

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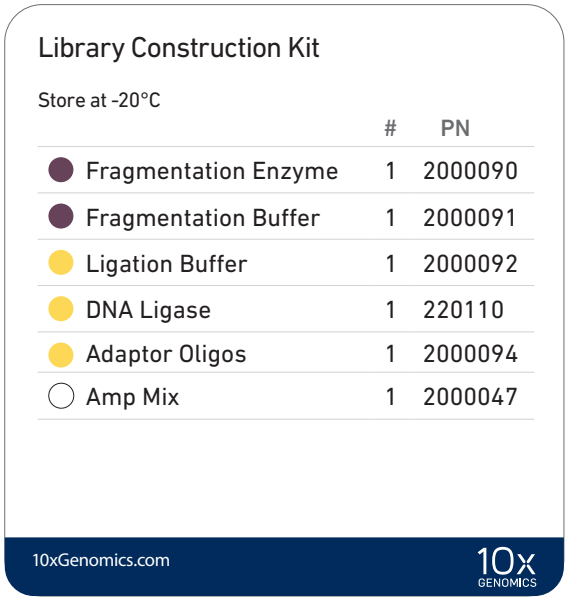
Library Construction Kit, 16 rxns PN-1000190 (store at –20°C)



Library Construction Kit

Store at -20°C

	#	PN
Fragmentation Enzyme	1	2000090
Fragmentation Buffer	1	2000091
Ligation Buffer	1	2000092
DNA Ligase	1	220110
Adaptor Oligos	1	2000094
Amp Mix	1	2000047

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Chromium Next GEM Single Cell Multiome Reagent Kit A, 4 rxns PN-1000284

Chromium Next GEM Single Cell Multiome GEM Kit A, 4 rxns PN-1000236 (store at –20°C)

Chromium
Next GEM Single Cell Multiome GEM Kit A
Store at -20°C

	#	PN
Barcoding Reagent Mix	1	2000267
Barcoding Enzyme Mix	1	2000273
Template Switch Oligo	1	3000228
Reducing Agent B	1	2000087
Cleanup Buffer	1	2000088
Quenching Agent	1	2000269

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell Multiome Amp Kit A, 4 rxns PN-1000237 (store at –20°C)

Chromium
Next GEM Single Cell Multiome Amp Kit A
Store at -20°C

	#	PN
Pre-Amp Mix	1	2000274
Pre-Amp Primers	1	2000271
Amp Mix	1	2000103
SI-PCR Primer B	1	2000128
cDNA Primers	1	2000089

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell Multiome Gel Bead Kit A, 4 rxns PN-1000235 (store at –80°C)

Chromium
Next GEM Single Cell Multiome Gel Beads A
Store at -80°C

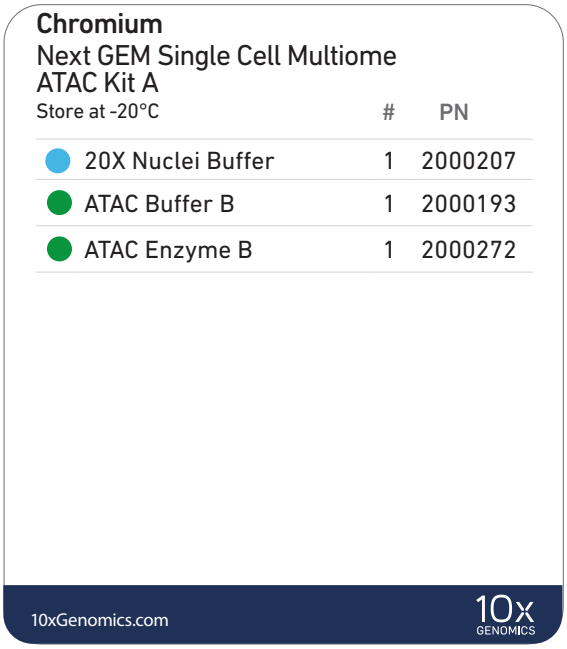
	#	PN
Single Cell Multiome Gel Beads A	1	2000261

10xGenomics.com 10x GENOMICS

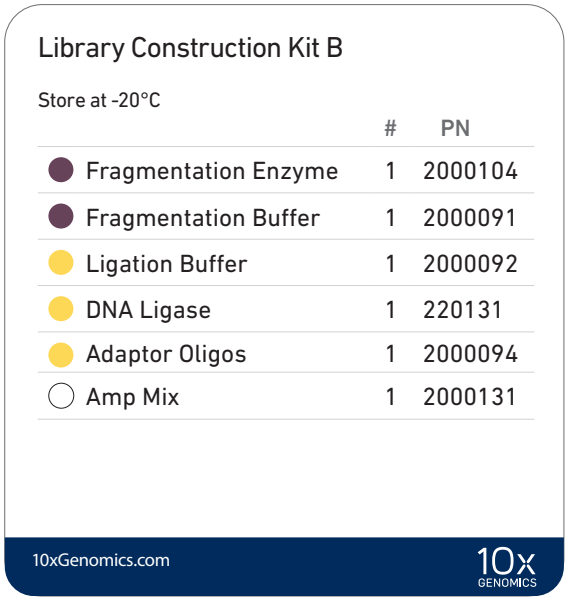
Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Single Cell Multiome ATAC Kit A, 4 rxns PN-1000281 (store at –20°C)



Library Construction Kit B, 4 rxns PN-1000279 (store at –20°C)



Chromium Next GEM Chip J Single Cell Kit, 48 rxns PN-1000234 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip J & Gaskets

	#	PN
Chromium Next GEM Chip J	6	2000264
Gasket, 6-pack	1	370017

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Chromium Next GEM Chip J Single Cell Kit, 16 rxns PN-1000230 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip J & Gaskets

	#	PN
Chromium Next GEM Chip J	2	2000264
Gasket, 2-pack	1	3000072

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10x GENOMICS

Single Index Kit N Set A, 96 rxns PN-1000212 (store at –20°C)

Single Index Kit N Set A

	#	PN
Single Index Plate N Set A	1	3000427

Dual Index Kit TT Set A, 96 rxns PN-1000215 (store at –20°C)

Dual Index Kit TT Set A

	#	PN
Dual Index Plate TT Set A	1	3000431

10x Genomics Accessories

Product	PN (Kit)	PN (Item)
10x Vortex Adapter	120251	330002
Chromium Next GEM Secondary Holder	1000195	3000332
10x Magnetic Separator*	120250	230003
10x Magnetic Separator B*	1000709 (Chromium X/iX Accessory Kit)/ 1000821 (Chromium X Series Accessory Kit)/ 1000707 (GEM-X Transition Kit)	2001212

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

Recommended Thermal Cyclers

Thermal cyclers used must support uniform heating of 100 µl emulsion volumes.

Supplier	Description	Part Number
Analytik Jena	Biometra TAdvanced 96 SG/S*	846-x-070-241/846-x-070-251 *(x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
Eppendorf	Mastercycler X50s/X50a**	6311000010 /6313000018
ThermoFisher	VeritiPro***	A48141
Bio-Rad	PTC Tempo Deepwell	12015392
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (discontinued)	1851197
Eppendorf	Mastercycler Pro (discontinued)	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler (discontinued)	4375786

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

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

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

****ThermoFisher VeritiPro requires FW 1.2.0, 96 well tray/retainer (PN 4381850), and "Cover Ramping" enabled*

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Single Cell protocols. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 N8010535
Rainin	Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR Tips LTS 20UL Filter RT-L10FLR	30389240 30389213 30389226
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	AM9937 12090-015
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous) Sigma Protector RNase Inhibitor DTT	E7023-500ML 3335399001 646563
Beckman Coulter	SPRIselect Reagent Kit	B23318
Bio-Rad	10% Tween 20	1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
Qiagen	Qiagen Buffer EB	19086
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Instrument & Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape/Reagents High Sensitivity D5000 ScreenTape/Reagents	G2939BA & G2953CA 5067-4626 G2991AA 5067-5584/5067-5585 5067-5592/5067-5593
Advanced Analytical	Fragment Analyzer Automated CE System - 12 cap Fragment Analyzer Automated CE System - 48/96 cap High Sensitivity NGS Fragment Analysis Kit	FSv2-CE2F FSv2-CE10F DNF-474
PerkinElmer	LabChip GX Touch HT Nucleic Acid Analyzer DNA High Sensitivity Reagent Kit	CLS137031 CLS760672
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms	KK4824

Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.

Choose Bioanalyzer, TapeStation, LabChip, or Fragment Analyzer based on availability & preference.

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin	**Pipettes**	
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
	Pipette Tips	
	Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf	**Pipettes**	
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µL	3125000010
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 10 – 100 µL	3125000036
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 100 – 300 µL	3125000052
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µL	3123000012
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µL	3123000020
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µL	3123000039
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µL	3123000055
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µL	3123000063
	Pipette Tips (compatible with Eppendorf pipettes only)	
	ep Dualfilter T.I.P.S., 2-20 µL	0030078535
	ep Dualfilter T.I.P.S., 2-200 µL	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µL	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1000 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20uL	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200uL	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1000uL	63300003

**Compatible with Rainin pipettes*

Protocol Steps & Timing

Steps		Timing	Stop & Store
Nuclei Isolation			
Dependent on Cell Type		~1-2 h	
Step 1 – Transposition			
1.1	Prepare Transposition Mix	10 min	
1.2	Isothermal Incubation	60 min	
Step 2 – GEM Generation & Barcoding			
2.1	Prepare Master Mix	10 min	
2.2	Load Chromium Next GEM Chip J	10 min	
2.3	Run the Chromium Controller or X Series	18 min	
2.4	Transfer GEMs	3 min	
2.5	GEM Incubation	75 min	
2.6	Quenching Reaction	5 min	STOP –80°C ≤ 4 week
Step 3 – Post GEM Incubation Cleanup			
3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	
Step 4 – Library Pre-Amplification PCR			
4.1	Prepare Pre-Amplification Mix	10 min	
4.2	Pre-Amplification PCR	30 min	STOP 4°C ≤18 h
4.3	SPRI Cleanup	15 min	STOP 4°C ≤72 h or –20°C long-term
Step 5 – Single Cell ATAC Library Construction			
5.1	Sample Index PCR	45 min	
5.2	Post Sample Index Double Sided Size Selection – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
5.3	Post Library Construction QC	60 min	
Step 6 – cDNA Amplification			
6.1	cDNA Amplification	40 min	STOP 4°C ≤72 h or –20°C ≤1 week
6.2	cDNA Cleanup – SPRIselect	15 min	STOP 4°C ≤72 h or –20°C ≤4 weeks
6.3	cDNA QC & Quantification	50 min	
Step 7 – Gene Expression Library Construction			
7.1	Fragmentation, End Repair & A-tailing	45 min	
7.2	Post Fragmentation, End Repair & A-tailing Double Sided – SPRIselect	30 min	
7.3	Adaptor Ligation	25 min	
7.4	Post Ligation Cleanup- SPRIselect	20 min	
7.5	Sample Index PCR	40 min	STOP 4°C ≤72 h
7.6	Post Sample Index PCR Double Sided – SPRIselect	30 min	STOP 4°C ≤72 h or -20°C long term
7.7	Post Library Construction QC	50 min	

Stepwise Objectives

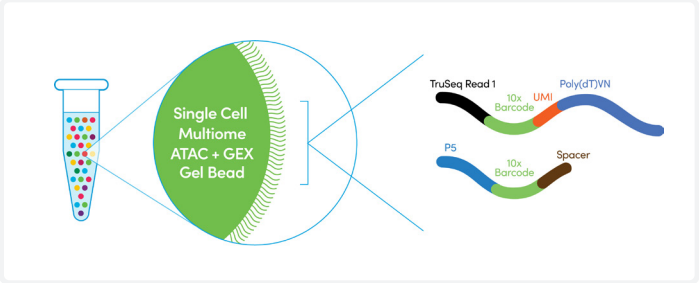
Chromium Single Cell Multiome + Gene Expression provides a comprehensive, scalable multiomic approach for simultaneously profiling epigenomic landscape and gene expression in the same single nuclei. This is achieved by transposing nuclei in a bulk solution. Using a microfluidic chip, the nuclei are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs). A pool of 736,000 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 Transposition

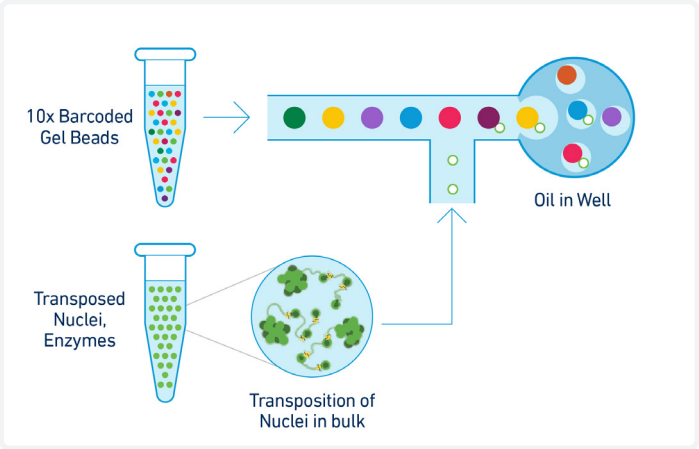
Nuclei suspensions are incubated in a Transposition Mix that includes a Transposase. The Transposase enters the nuclei and preferentially fragments the DNA in open regions of the chromatin. Simultaneously, adapter sequences are added to the ends of the DNA fragments.

Step 2 GEM Generation & Barcoding

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



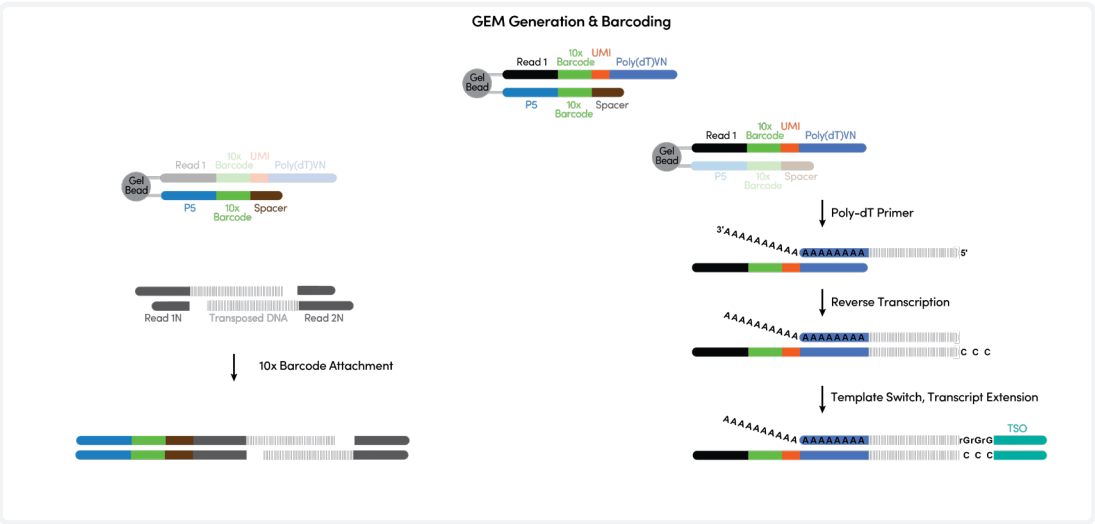
GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip J. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the 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 3 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 4 Pre-Amplification PCR

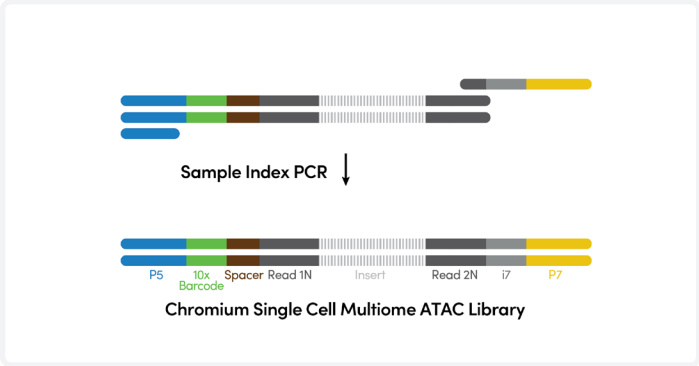
Barcoded transposed DNA and barcoded full length cDNA from poly-adenylated mRNA are amplified via PCR to fill gaps and for generating sufficient mass for library construction. The pre-amplified product is used as input for both ATAC library construction and cDNA amplification for gene expression library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

Pooled pre-amplification PCR

Step 5 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® bridge amplification.

ATAC Library Construction



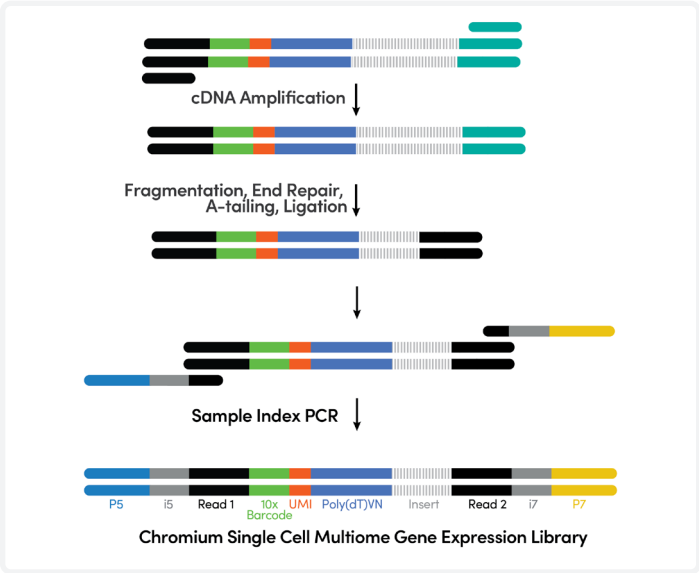
Step 6 cDNA Amplification

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

Step 7 Gene Expression Library Construction

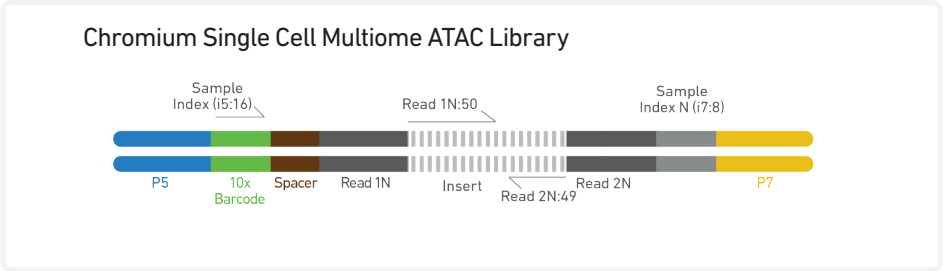
Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. P5, P7, i7 and i5 sample indexes, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final gene expression libraries contain the P5 and P7 primers used in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

cDNA Amplification &
Gene Expression Library Construction

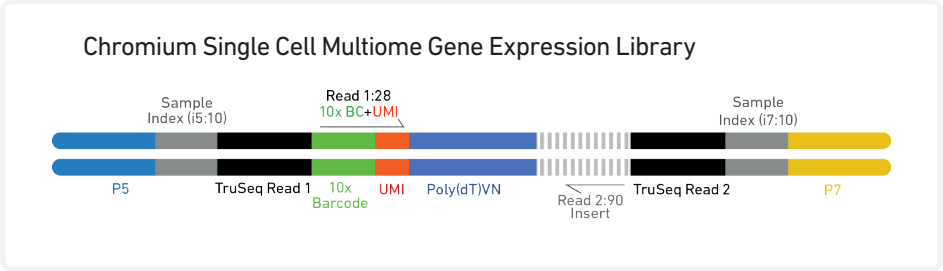


Step 8 Sequencing

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 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.



Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Emulsion-safe Plastics

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

Multiplet Rate

Multiplet Rate (%)	# of Nuclei Loaded	# of Nuclei Recovered
0.4%	~775	~500
0.8%	~1,550	~1,000
1.6%	~3,075	~2,000
2.3%	~4,625	~3,000
3.1%	~6,150	~4,000
3.9%	~7,700	~5,000
4.6%	~9,250	~6,000
5.4%	~10,750	~7,000
6.2%	~12,300	~8,000
6.9%	~13,850	~9,000
7.7%	~15,400	~10,000

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.
- Prepare 50% glycerol solution:
  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
  ii. Filter through a 0.2 µm filter.
  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

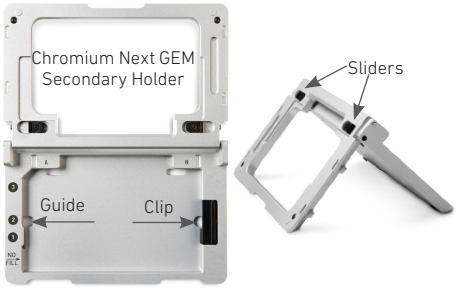
- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium Next GEM 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 multiple chips are to be used, 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 bottom 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 either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- 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.

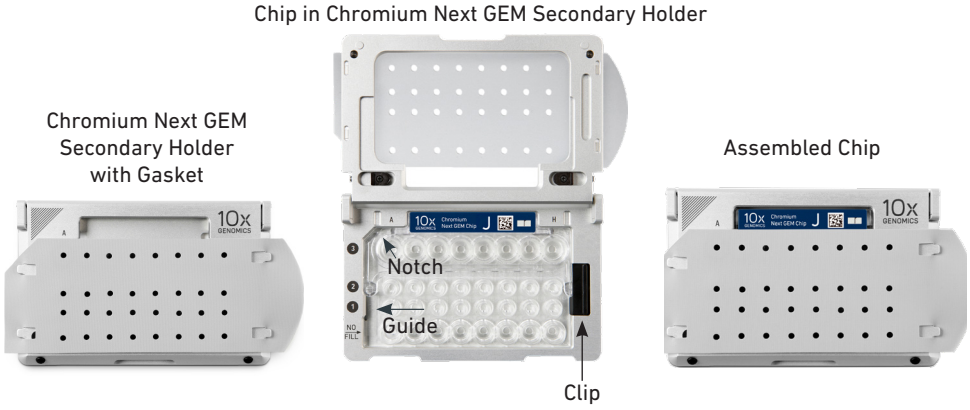
Chromium Next GEM Secondary Holders

- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.



Chromium Next GEM 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 into the wells.



Chromium Next GEM 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 Chromium Next GEM Chip J for specific instructions.

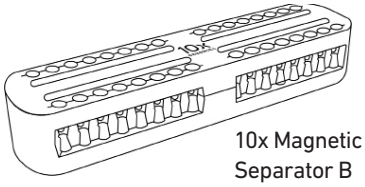
Gel Bead Handling

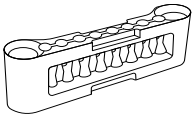
- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.

- 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 the tubes and the liquid levels look even. Place the 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.

10x Magnetic Separator

- Images below are illustrative - 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.

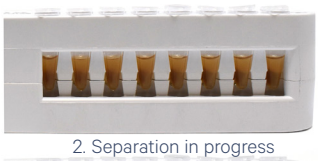

10x Magnetic Separator B


10x Magnetic Separator

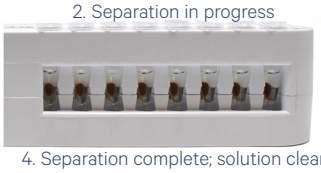
Magnetic Bead Cleanup Steps

- 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 adjacent panel for an example.
- The time need for the solution to clear may vary based on specific step, reagents, volume of reagents used 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

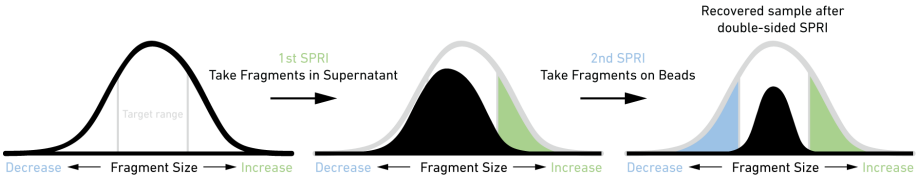
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

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

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

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

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

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

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 interchangebaly.

Step 1

Transposition

1.1 Prepare Transposition Mix

1.2 Isothermal Incubation

1.0 Transposition

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	🟢	**ATAC Buffer B**	2000193	Vortex, centrifuge briefly.	–20°C
	🔵	**20X Nuclei Buffer*** *Concentrated 20X stock; dilute 1:20 in nuclease-free water before use. (See below to Prepare Diluted Nuclei Buffer)	2000207	Thaw. Vortex, centrifuge briefly.	–20°C
Place on Ice	🟢	**ATAC Enzyme B**	2000265/ 2000272	Centrifuge briefly.	–20°C
		Nuclei** in Diluted Nuclei Buffer (See below to Prepare Diluted Nuclei Buffer)			

**Refer to Demonstrated Protocols for Nuclei Isolation for ATAC + Gene Expression Sequencing (Documents CG000365, CG000366, CG000375, CG000505). Adhering to these protocols is recommended for optimal assay performance.

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.

Prepare **Diluted Nuclei Buffer**

Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
🔵 20X Nuclei Buffer (PN-2000207)	20X	1X	50 µl
DTT	1,000 mM	1 mM	1 µl
RNase Inhibitor (confirm vendor-specific stock concentration)	40 U/µl	1 U/µl	25 µl
Nuclease-free Water	-	-	924 µl

Nuclei Concentration Guidelines

Based on the Targeted Nuclei Recovery, resuspend the nuclei in Diluted Nuclei Buffer to get corresponding Nuclei Stock Concentrations (see Table). This enables pipetting volumes of the Nuclei Stock for Transposition (step 1.1) to be 2-5 µl. Higher Nuclei Stock Concentrations will result in lower pipetting volumes that may increase nuclei input variability.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	160-400
1,000	320-810
2,000	650-1,610
3,000	970-2,420
4,000	1,290-3,230
5,000	1,610-4,030
6,000	1,940-4,840
7,000	2,260-5,650
8,000	2,580-6,450
9,000	2,900-7,260
10,000	3,230-8,060

Calculate volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µl

$$\text{Volume of Nuclei Stock (µl)} = \frac{\text{Targeted Nuclei Recovery} \times 1.61 \text{ (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ µl)}}$$

Volume of Diluted Nuclei Buffer* (µl) = **5 µl** - volume of Nuclei Stock (µl)

*Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water)

Example Calculation

Targeted Nuclei Recovery = 4000 nuclei

Nuclei Stock Concentration = 2500 nuclei/µl

Recovery efficiency factor 1.61

Volume of Nuclei Stock (µl) =

$$\frac{\text{Targeted Nuclei Recovery} \times 1.61 \text{ (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/µl)}} = \frac{4000 \times 1.61}{2500} = \mathbf{2.58\ µl}$$

Volume of Diluted Nuclei Buffer = 5 µl - 2.58 µl = **2.42 µl**

Add calculated volumes of Diluted Nuclei Buffer and Nuclei Stock to the Transposition Mix in step 1.1

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 B	2000193	7.0	30.8	61.6
ATAC Enzyme B	2000265/ 2000272	3.0	13.2	26.4
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 Nuclei Concentration Guidelines to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of **5 µl**.

d. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix. 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 6x (pipette set to 10 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	15 µl	60 min
Step	**Temperature**	**Time**
Incubate	37°C	00:60:00
Hold	4°C	Hold

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

Step 2

GEM Generation & Barcoding

2.1 Prepare Master Mix

2.2 Load Chromium Next GEM Chip J

2.3 Run the Chromium Controller or X Series

2.4 Transfer GEMs

2.5 GEM Incubation

2.6 Quenching Reaction

2.0 GEM Generation & Barcoding

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Single Cell Multiome Gel Beads**	2000261	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	●	**Template Switch Oligo**	3000228	Centrifuge briefly, resuspend in 80 µl Low TE Buffer. Vortex 15 sec at maximum speed, centrifuge briefly, leave at room temperature for ≥ 30 min. 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 Mix**	2000267	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	●	**Barcoding Enzyme Mix**	2000266/ 2000273	Maintain on ice. Store at –20°C immediately after use.	–20°C
Obtain	●	**Partitioning Oil**	2000190	-	Ambient
		Low TE Buffer	-	Manufacturer's recommendations.	-
		Chromium Next GEM Chip J	2000264	See Tips & Best Practices.	Ambient
		10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
		10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
		Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices	Ambient
		50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the protocol.

2.1 Prepare Master Mix

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

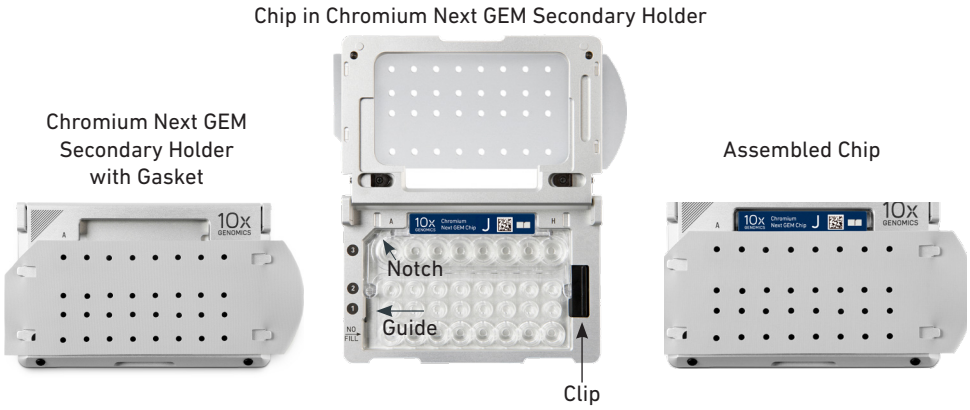
	Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
●	**Barcoding Reagent Mix**	2000267	49.5	217.8	435.6
●	**Template Switch Oligo**	3000228	1.1	4.8	9.7
○	**Reducing Agent B**	2000087	1.9	8.4	16.7
●	**Barcoding Enzyme Mix**	2000266/ 2000273	7.5	33.0	66.0
	Total	-	**60.0**	**264.0**	**528.0**

Assemble Chromium Next GEM Chip

TIPS

See Tips & Best Practices for chip handling instructions.

- 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.
- Remove the chip from the sealed bag. Use the chip within ≤ 24 h.
- 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 open until ready for and while dispensing reagents into the wells. DO NOT touch the smooth side of the gasket. After loading reagents, close the chip holder. DO NOT press down on the top of the gasket.



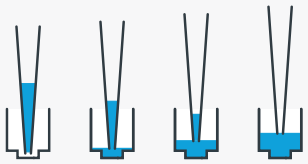
For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 2.2 for details.

2.2 Load Chromium Next GEM Chip J

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.*

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 pipette centered to each well and* the tips slightly submerged.

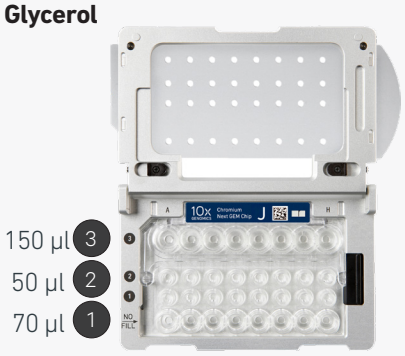


a. Add 50% glycerol solution to each unused well
(if processing <8 samples/chip)

- **70 µl** in each unused well in row labeled **1**
- **50 µl** in each unused well in row labeled **2**
- **150 µl** in each unused well in row labeled **3**

DO NOT add 50% glycerol solution to the bottom row of NO FILL wells.
DO NOT use any substitute for 50% glycerol solution.

Glycerol



b. Prepare Master Mix + Transposed Nuclei

- Add **60 µl** Master Mix to each tube containing Transposed Nuclei for a total of **75 µl** in each tube.

c. Load Row Labeled 1

- Gently pipette mix the Master Mix + Transposed Nuclei 5x
- Using the same pipette tip, dispense **70 µl** Master Mix + Transposed Nuclei into the bottom center of each well in **row labeled 1** without introducing bubbles.

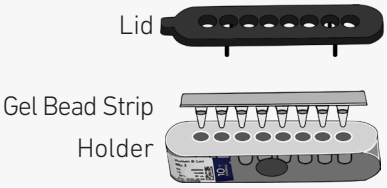
Master Mix + Transposed Nuclei

The illustrated chip is being loaded for 8 samples.

d. 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 and the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.

Prep Gel Beads



e. Load Row Labeled 2

- Puncture the foil seal of the Gel Bead tubes.
- Slowly aspirate **50 µl** Gel Beads.
- Dispense into the wells in **row labeled 2** without introducing bubbles.
- Wait **30 sec.**

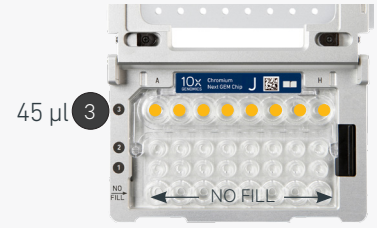
Gel Beads

f. Load Row Labeled 3

- Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir.

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

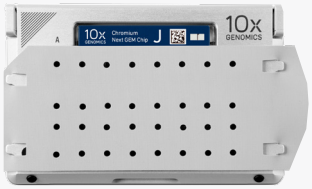
Partitioning Oil



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.

  Run the chip in the Chromium instrument immediately after loading the Partitioning Oil



Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

2.3 Run the Chromium Controller or X Series

If using Chromium Controller:

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

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 **(~18 min)**, the Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.00 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the protocol.

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

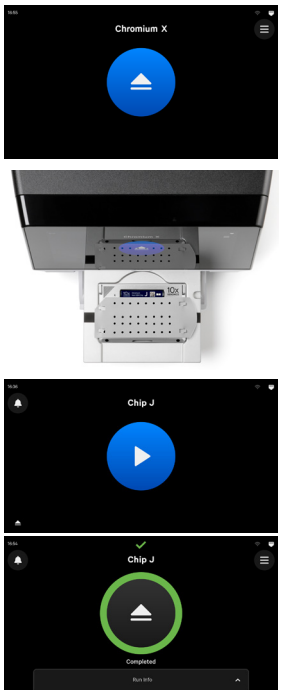
If using Chromium X Series:

a. Press the eject button on Chromium 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 (~18 min), the instrument will chime. **Immediately** proceed to the next step.



2.4 Transfer GEMs

a. Place a PCR 8-tube strip on ice.

b. Press the eject button of the Controller to remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

Expose Wells at 45 Degrees

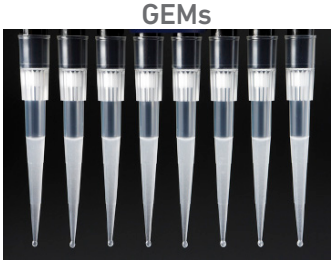
d. Visually compare the remaining volume in rows labeled 1-2. Abnormally high volume in one well relative to other wells may indicate a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the pipette tips and the wells.

Transfer GEMs

f. 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.

GEMs



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

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip or plate and place on ice for no more than **1 h**.

2.5 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.

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 (not overnight*)

Retrieve Quenching Agent (● PN-2000269) from **-20°C** and equilibrate to **room temperature** while the PCR program is running.

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

2.6 Quenching Reaction

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

b. Slowly pipette mix 10x (pipette set to 90 µl). The solution will be viscous. Ensure that no liquid remains along the tube sidewalls and pipette tips. If necessary, aspirate the entire volume and dispense back slowly into the tube.

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

Step 3

Post GEM Incubation Cleanup

3.0 Post GEM Incubation Cleanup

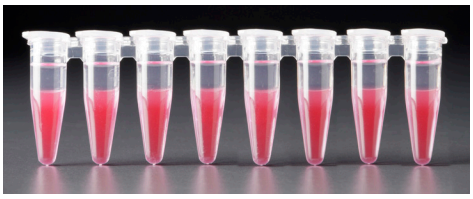
GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	◯	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Nuclease-free Water	-	-	-
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥**30 sec**) to resuspend beads immediately 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/ 10x Magnetic Separator B	230003/ 2001212	-	Ambient
		Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh in nuclease free water.	-

3.1 Post GEM Incubation Cleanup – Dynabeads

a. Add **125 µl** Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Gently invert tube 10x to mix. Centrifuge briefly.

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

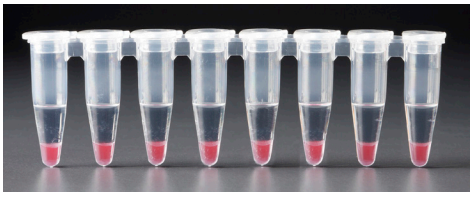
Biphasic Mixture



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.

Remove Recovery Agent



c. Prepare Dynabeads Cleanup Mix.

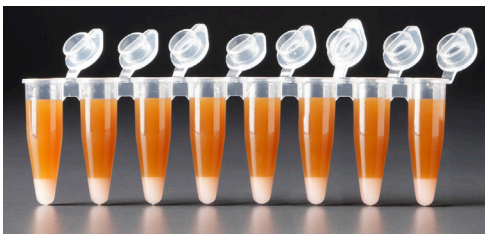
Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Cleanup Buffer	2000088	182.0	801.0	1,602.0
Dynabeads MyOne SILANE Vortex thoroughly (≥**30 sec**) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	13.0	57.2	114.4
Reducing Agent B	2000087	5.0	22.0	44.0
Total	-	**200.0**	**880.2**	**1760.4**

Resuspend clump

d. Vortex and add **200 µl** Dynabeads Cleanup Mix to each sample. Pipette mix 10x (pipette set to 200 µl).

e. Incubate **10 min** at **room temperature** (keep caps open).

Add Dynabeads Cleanup Mix



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

Elution Solution I* *Add reagents in the order listed*	PN	1X (µl)	4X + 15% (µl)	8X + 15% (µl)
Buffer EB	-	98.0	450.8	901.6
10% Tween 20	-	1	4.6	9.2
Reducing Agent B	200087	1	4.6	9.2
Total	-	**100**	**460**	**920**

TIPS

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

h. Remove the supernatant.

i. Add **300 µl** freshly prepared 80% ethanol to the pellet while on the magnet•**High**. 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 magnet•**Low**.

n. Remove remaining ethanol.

o. Remove from the magnet. **Immediately** add **50.5 µl** Elution Solution I (prepared in step 3.1f) to avoid clumping.

p. Pipette mix (pipette set to 40 µl) without introducing bubbles.

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

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

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

3.2 Post GEM Incubation Cleanup – SPRIselect

a. Vortex the SPRIselect reagent until fully resuspended. Add **90 µl** SPRIselect reagent to each sample. Pipette mix 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. **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 4

Pre-Amplification PCR

4.1 Prepare Pre-Amplification Mix

4.2 Pre-Amplification PCR

4.3 SPRI Cleanup

4.0 Pre-Amplification PCR

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	Gently pipette mix, centrifuge briefly.	–20°C
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator/ 10x Magnetic Separator B	230003/ 2001212	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	Ambient

4.1 Prepare Pre-Amplification Mix

a. Prepare Pre-Amplification 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)
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.

4.2 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 6X (Total 7 cycles)
6	72°C	00:01:00
7	4°C	Hold

STOP

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

4.3 Pre-Amplification SPRI Cleanup

a. Vortex the SPRIselect reagent until fully resuspended. Add **160 µl** SPRIselect reagent to each sample. Pipette mix 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 **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. **Immediately** add **160.5 µl** Buffer EB.

l. Pipette mix (pipette set to 150 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 **160 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.

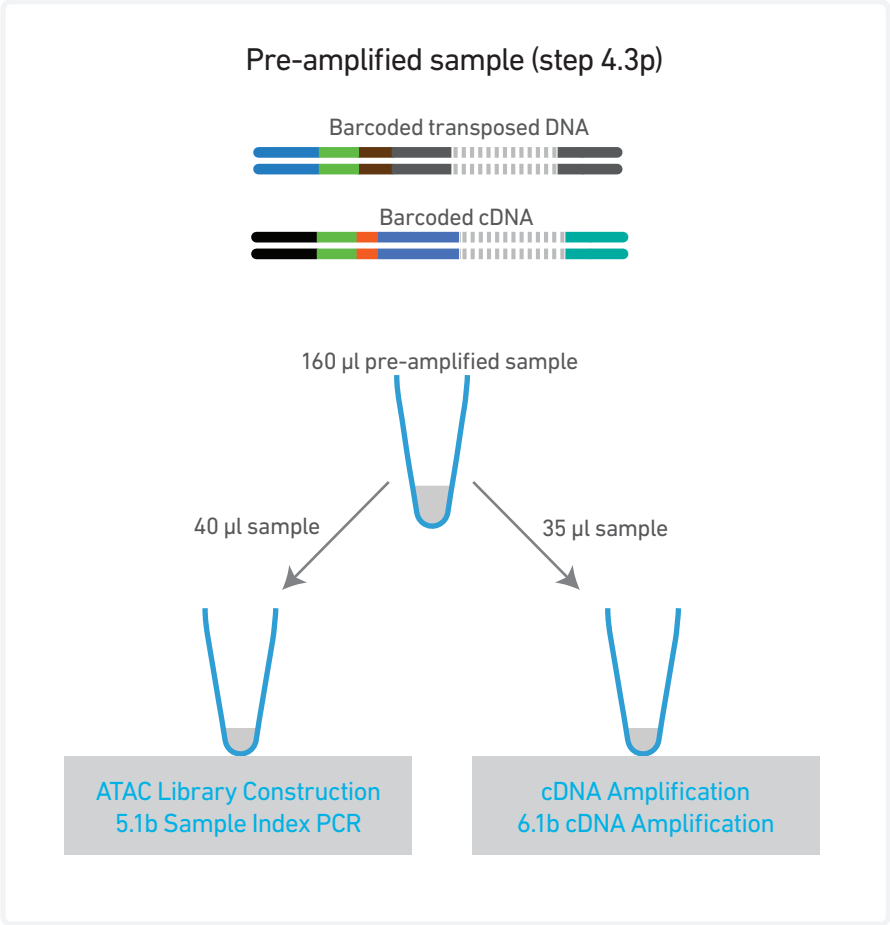
Sample Split Overview

- **160 µl pre-amplified, SPRI cleaned** sample derived at step 4.3p 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 5)

  II. 35 µl sample is used for cDNA Amplification (step 6).
  The amplified cDNA will be used for Gene Expression Library Construction.

Store the remaining pre-amplification product at **–20°C** long term for generating additional libraries.



Step 5

ATAC Library Construction

5.1 Sample Index PCR

5.2 Post Sample Index Double Sided Size Selection – SPRIselect

5.3 Post Library Construction QC

5.0 ATAC Library Construction

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/ 2000103	Vortex, centrifuge briefly.	–20°C
		KAPA Library Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations.	-
Obtain		**Qiagen Buffer EB**	-	-	Ambient
		10x Magnetic Separator/ 10x Magnetic Separator B	230003/ 2001212	See Tips & Best Practices.	Ambient
		Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	Ambient

5.1 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.

Sample Index PCR Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
○ **Amp Mix**	2000047/ 2000103	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 4.3p to a new tube strip (**35 µl** of the remaining sample volume will be used for cDNA Amplification and the rest can be stored at **–20°C** long term for generating additional libraries). 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 for based on Targeted Nuclei Recovery.

Cycle Number Optimization Table

Targeted Nuclei Recovery	Total Cycles
≤2,000	9
2,001-6,000	8
6,001-10,000	7

STOP

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

5.2 Post Sample Index Double Sided Size Selection – SPRIselect

TIPS

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

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.

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.

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.

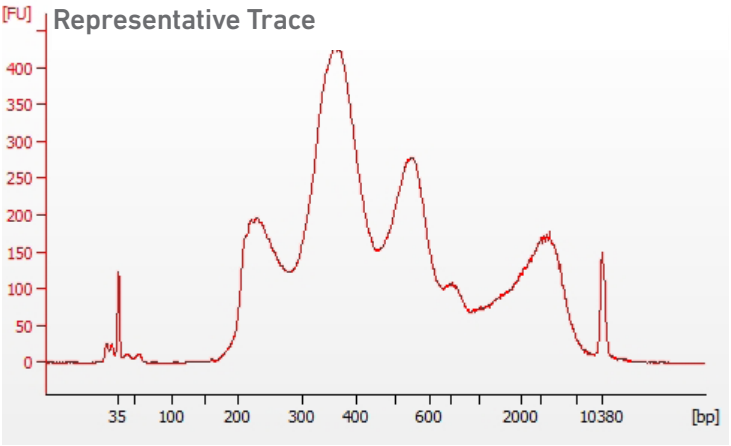
r. Transfer **20 µl** sample to a new tube strip.

STOP

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

5.3 Post Library Construction QC

a. Run **1 µl** sample 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
- LabChip

See Appendix for Post Library Construction Quantification

Step 6

cDNA Amplification

6.1 cDNA Amplification

6.2 cDNA Cleanup – SPRIselect

6.3 cDNA QC & Quantification

6.0 cDNA Amplification

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.	-
Place on ice	○	**Amp Mix**	2000047/ 2000103	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.	-	-	-

6.1 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/ 2000103	50	220	440
● **cDNA Primers**	2000089	15	66	132
Total	-	**65**	**286**	**572**

b. Transfer **35 µl** pre-amplified sample from step 4.3p to a new tube strip (store the remaining pre-amplification product at **–20°C** long term for generating additional libraries). 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
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

STOP

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

6.2 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**.

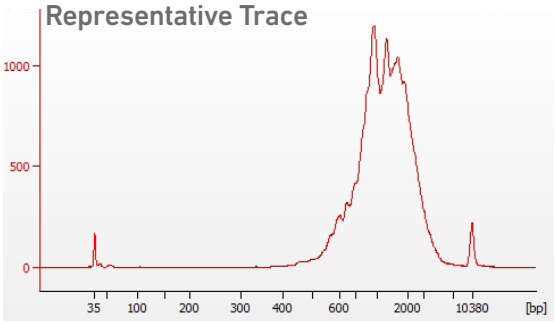
l. Place the tube strip on the magnet•**High** until the solution clears.

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.

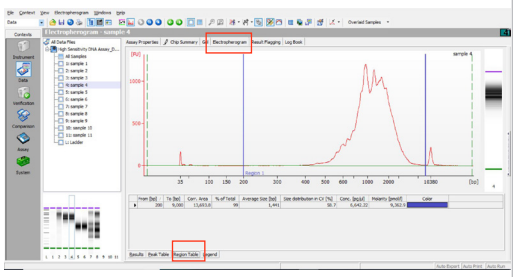
6.3 cDNA QC & Quantification

a. Run 1 µl undiluted sample on an Agilent Bioanalyzer High Sensitivity chip.
For input cells with low RNA content (<1 pg total RNA/cell), 1 µl undiluted product may be run.
Lower molecular weight product (35 – 150 bp) may be present. This is normal and does not affect sequencing or application performance.

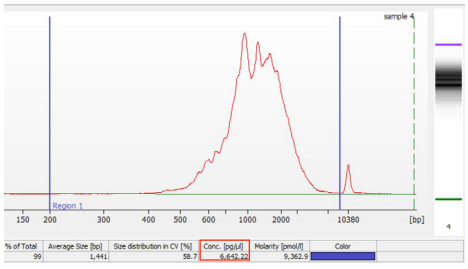


EXAMPLE CALCULATION

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

$$= \frac{\text{Conc'n (pg/µl)} \times \text{Elution Volume (µl)} \times \text{Dilution Factor}}{1000 \text{ (pg/ng)}}$$

$$= \frac{6642.22 \text{ (pg/µl)} \times 40 \text{ (µl)} \times 1}{1000 \text{ (pg/ng)}} = 265.69 \text{ ng}$$

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

= 0.25 x Total cDNA yield

= 0.25 x 265.69= 66.42 ng

Refer to step 7.5 for appropriate number of Sample Index PCR cycles based on carry forward cDNA yield/input cDNA.

Alternate Quantification Methods (See Appendix for representative traces)

- Agilent TapeStation
- LabChip

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

Step 7

Gene Expression Library Construction

7.0 Gene Expression Library Construction

GET STARTED!

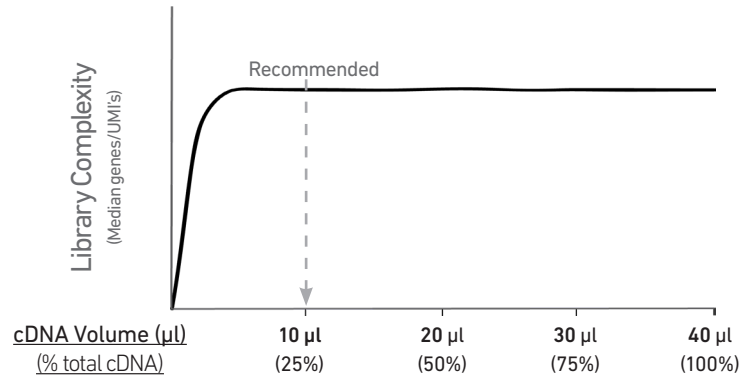
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	●	**Fragmentation Buffer**	2000091	Vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Adaptor Oligos**	2000094	Vortex, centrifuge briefly.	–20°C
	●	**Ligation Buffer**	2000092	Vortex, verify no precipitate, centrifuge briefly.	–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	Centrifuge briefly.	–20°C
	●	**DNA Ligase**	220110/ 220131	Centrifuge briefly.	–20°C
	○	**Amp Mix**	2000047/ 2000131	Vortex, centrifuge briefly.	–20°C
		KAPA Library Quantification Kit for Illumina Platforms	-	Manufacturer's recommendations.	-
Obtain		**Qiagen Buffer EB**	-	-	Ambient
		10x Magnetic Separator/ 10x Magnetic Separator B	230003/ 2001212	See Tips & Best Practices.	Ambient
		Prepare 80% Ethanol Prepare 20 ml for 8 reactions	-	Prepare fresh.	Ambient

Step Overview (Step 7.1d)

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 6.2n. 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**).

Correlation: cDNA input & Library Complexity



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 7.5d) 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 6.3).

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

7.1 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**
Pre-cool block Pre-cool block prior to preparing the Fragmentation Mix	4°C	Hold
Fragmentation	32°C	00:05:00
End Repair & A-tailing	65°C	00:30:00
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)
Fragmentation Buffer	2000091	5	22	44
Fragmentation Enzyme	2000090/ 2000104	10	44	88
Total	-	**15**	**66**	**132**

d. Transfer **ONLY 10 µl** purified cDNA sample from cDNA Cleanup (step 6.2n) 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 **25 µl** Buffer EB to each sample.

f. Add **15 µl** Fragmentation Mix to each sample.

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

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

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

m. Remove from the magnet. Add **50.5 µl** Buffer EB to each sample. Pipette mix 15x (pipette set to 45 µl) .

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.

7.3 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 Buffer	2000092	20	88	176
DNA Ligase	220110/ 220131	10	44	88
Adaptor Oligos	2000094	20	88	176
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

7.4 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.

7.5 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** Amp Mix (PN-2000047/2000131) 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 Post cDNA Amplification QC & Quantification (step 6.3)

Recommended cycle numbers

cDNA Input	Total Cycles
0.25-25 ng	14-16
25-150 ng	12-14
150-500 ng	10-12
500-1,000 ng	8-10
1,000-1,500 ng	6-8
>1500 ng	5

STOP

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

7.6 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 (pipette set to 35 µl).

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

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

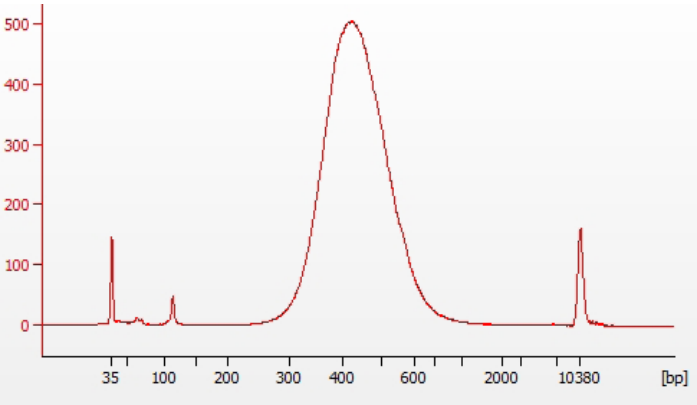
p. Transfer **35 µl** to a new tube strip.

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

7.7 Post Library Construction QC

Run **1 µl s**ample at **1:3 dilution** on an Agilent Bioanalyzer High Sensitivity chip.

Representative Trace



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
- LabChip

See Appendix for Post Library Construction Quantification

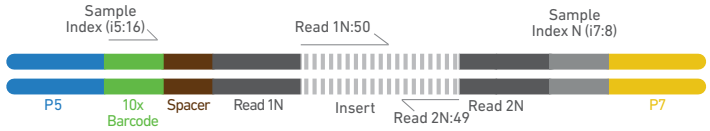
Sequencing

8

Sequencing Libraries

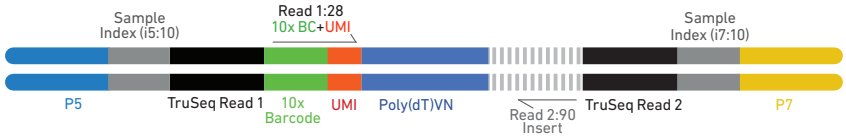
Chromium Single Cell Multiome ATAC libraries comprise double stranded DNA with standard Illumina® paired-end constructs which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder that includes paired-end Read 1N and Read 2N used for sequencing 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 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



Illumina® Sequencer Compatibility

The compatibility of the listed sequencers has been verified by 10x Genomics. Some variation in assay performance is expected based on sequencer choice. For more information about performance variation, visit the 10x Genomics Support website.

- MiSeq™
- NextSeq™ 500/550 (High Output)
- NextSeq™ 1000/2000
- HiSeq 2500™ (Rapid Run)
- HiSeq™ 3000/4000
- NovaSeq™ 6000

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, ATAC libraries should be denatured and diluted according to the table below. Consult the Technical Note on Sequencing Metrics and Base Composition of Single Cell Multiome ATAC Libraries (CG000373), available at 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 workflow	150	1
*NovaSeq™ 6000 Xp workflow	150	1

** The NovaSeq 6000 standard workflow permits loading one library pool across all lanes of the flow cell; whereas the Xp workflow enables sequencing various library pools in each lane of the NovaSeq 6000 flow cell. For more information on both workflows, refer to the NovaSeq 6000 Denature and Dilute Guide.*

Library quantification should be done with the KAPA DNA Quantification Kit using the average insert size determined by Agilent Bioanalyzer, Perkin Elmer LabChip, or Agilent TapeStation QC. Alternate methods to KAPA qPCR for final library quantification may result in under quantification, and consequently overloading.

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 according to the table below. Refer to Illumina® documentation for denaturing and diluting libraries. As the Multiome Gene Expression library is same as the Chromium Single Cell 3' Gene Expression Dual Index library, consult the Technical Note on Sequencing Metrics & Base Composition of Single Cell 3' v3.1 Dual Index Libraries (CG000374), available at the 10x Genomics Support website, for more information.

Library quantification should be done with the KAPA DNA Quantification Kit using the average insert size determined by Agilent Bioanalyzer, Perkin Elmer LabChip, or Agilent TapeStation QC. Alternate methods to KAPA qPCR for final library quantification may result in under quantification, and consequently overloading.

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 workflow	150	1
*NovaSeq™ 6000 Xp workflow	150	1

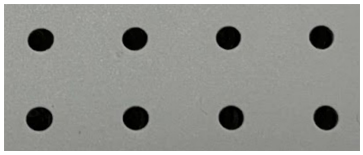
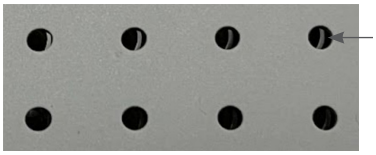
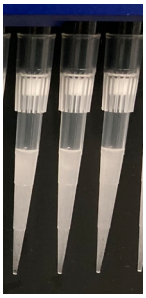
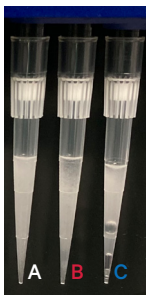
**The NovaSeq 6000 standard workflow permits loading one library pool across all lanes of the flow cell; whereas the Xp workflow enables sequencing various library pools in each lane of the NovaSeq 6000 flow cell. For more information on both workflows, refer to the NovaSeq 6000 Denature and Dilute Guide.*

Library Pooling

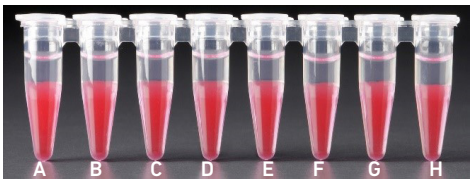
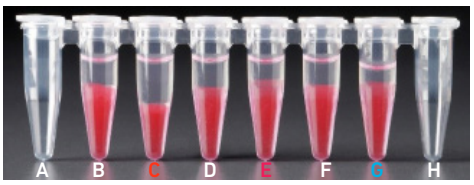
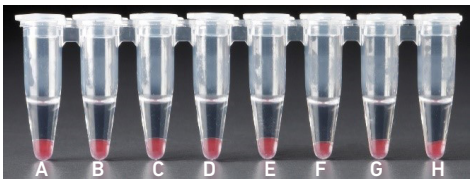
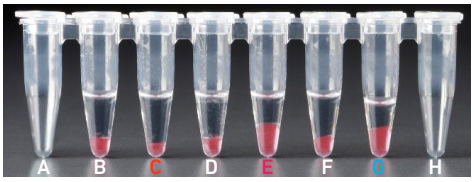
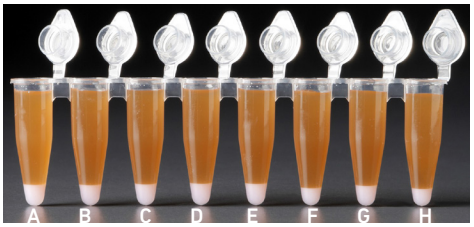
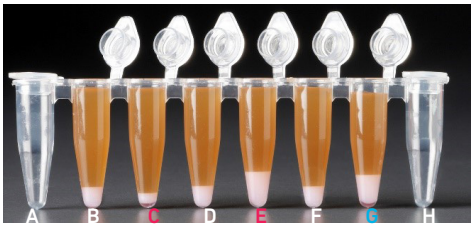
Single Cell Multiome ATAC libraries maybe pooled with other ATAC libraries only when using forward strand Illumina® workflow. Single Cell Multiome Gene Expression libraries maybe pooled for sequencing with other libraries, taking into account the differences in cell number and per-cell read depth requirements 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.

Troubleshooting

GEM Generation & Barcoding

STEP	NORMAL	IMPACTED
2.2 Load Chromium Next GEM 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.
2.4 d After Chip J is removed from the Controller or X/iX and the wells are exposed	 All 8 recovery wells are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
2.4 e Transfer GEMs from Chip J Row Labeled 3	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Adequate emulsion volume (no clog or wetting failure) Wetting failure Low emulsion volume (clog) Pipette tip A shows normal GEM generation, pipette tip B indicates a wetting failure, and pipette tip C shows a clog and wetting failure.

Consult the Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures (Technical Note CG000479) for more information.

STEP	NORMAL	IMPACTED
3.1 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).
3.1 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).
3.1 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).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to 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. Consult the Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures (Technical Note CG000479) for more information.

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Error Detected: Row _ Pressure:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket, open and close the lid to ensure the gasket is properly aligned, and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

e. **Chip Holder Not Present:** Open the controller drawer and check if chip holder is present. Insert chip properly into chip holder and retry.

f. **Unauthorized Chip:** This indicates that an incompatible non-Next GEM chip has been used with an instrument that only can run Next GEM assays. Use only Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263;120212) to run that chip or chip must be discarded. Contact support@10xgenomics.com for further assistance.

g. **Endpoint Reached Early:** If this message is received, contact support@10xgenomics.com for further assistance.

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

Appendix

Post Library Construction Quantification
Agilent TapeStation Traces
LabChip Traces
Assay Scheme Overview
Sequences

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 insert size derived from the Bioanalyzer/TapeStation trace.

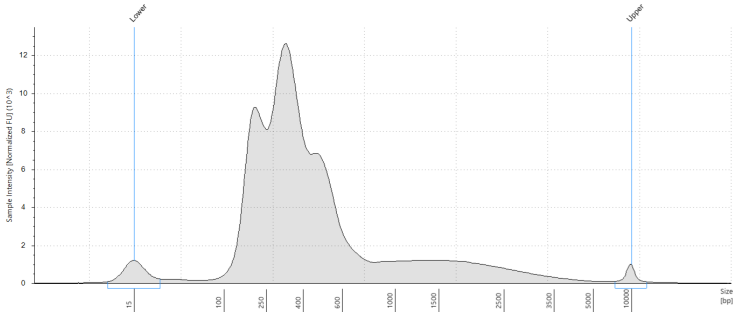
Agilent TapeStation Traces

Agilent TapeStation Traces

Agilent TapeStation High Sensitivity D5000 ScreenTape was used.
Protocol steps correspond to the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338).

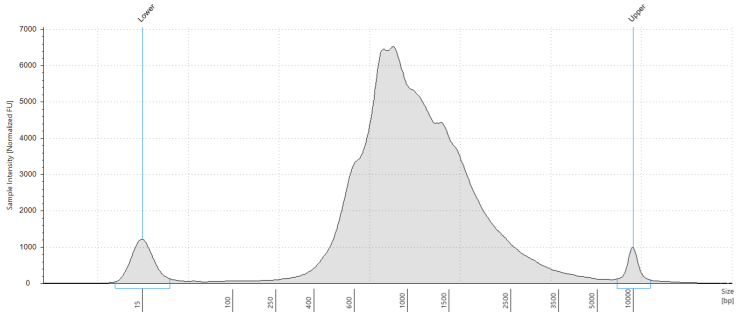
Protocol Step 5.3 – Post Library Construction QC (ATAC Library)

Run 2 µl sample



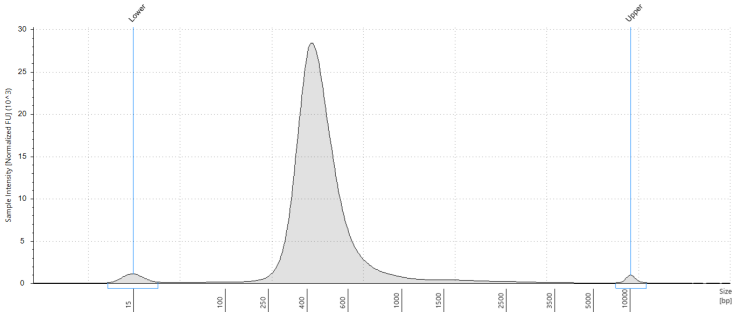
Protocol Step 6.3 – cDNA QC & Quantification

Run 2 µl sample



Protocol Step 7.7 – Post Library Construction QC (Gene Expression Library)

Run 2 µl sample



All traces are representative.

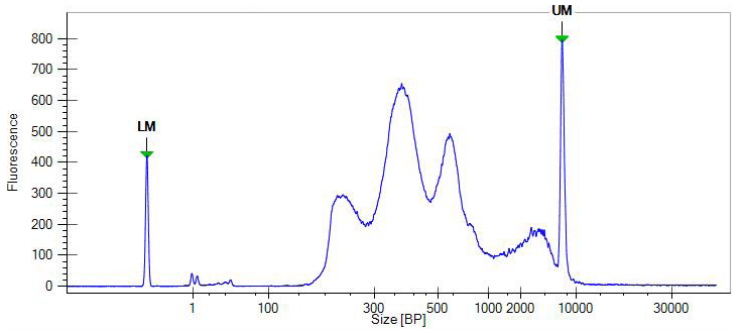
LabChip Traces

LabChip Traces

DNA High Sensitivity Reagent Kit was used. Protocol steps correspond to the Chromium Next GEM Single Cell Multiome ATAC + GEX User Guide (CG000338).

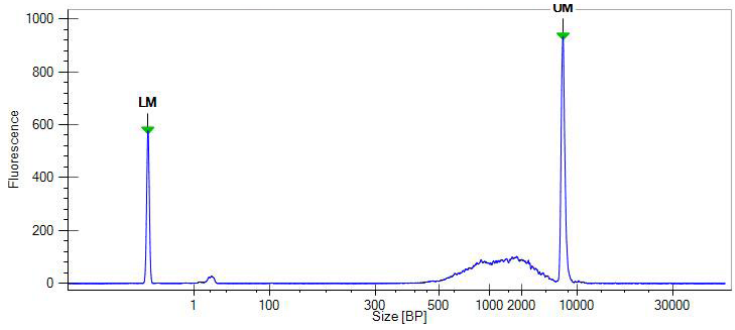
Protocol Step 5.3 – Post Library Construction QC (ATAC Library)

Run 10 µl undiluted sample



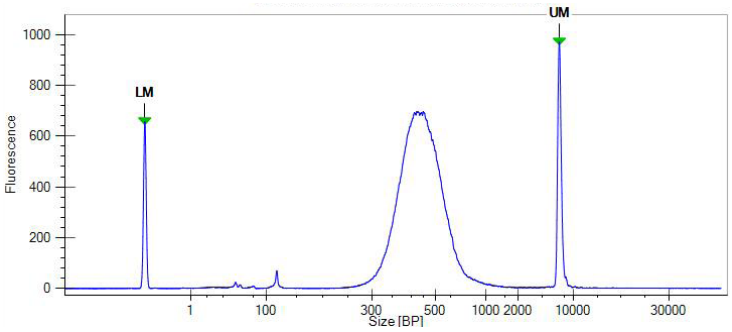
Protocol Step 6.3 – cDNA QC & Quantification

Run 10 µl undiluted sample



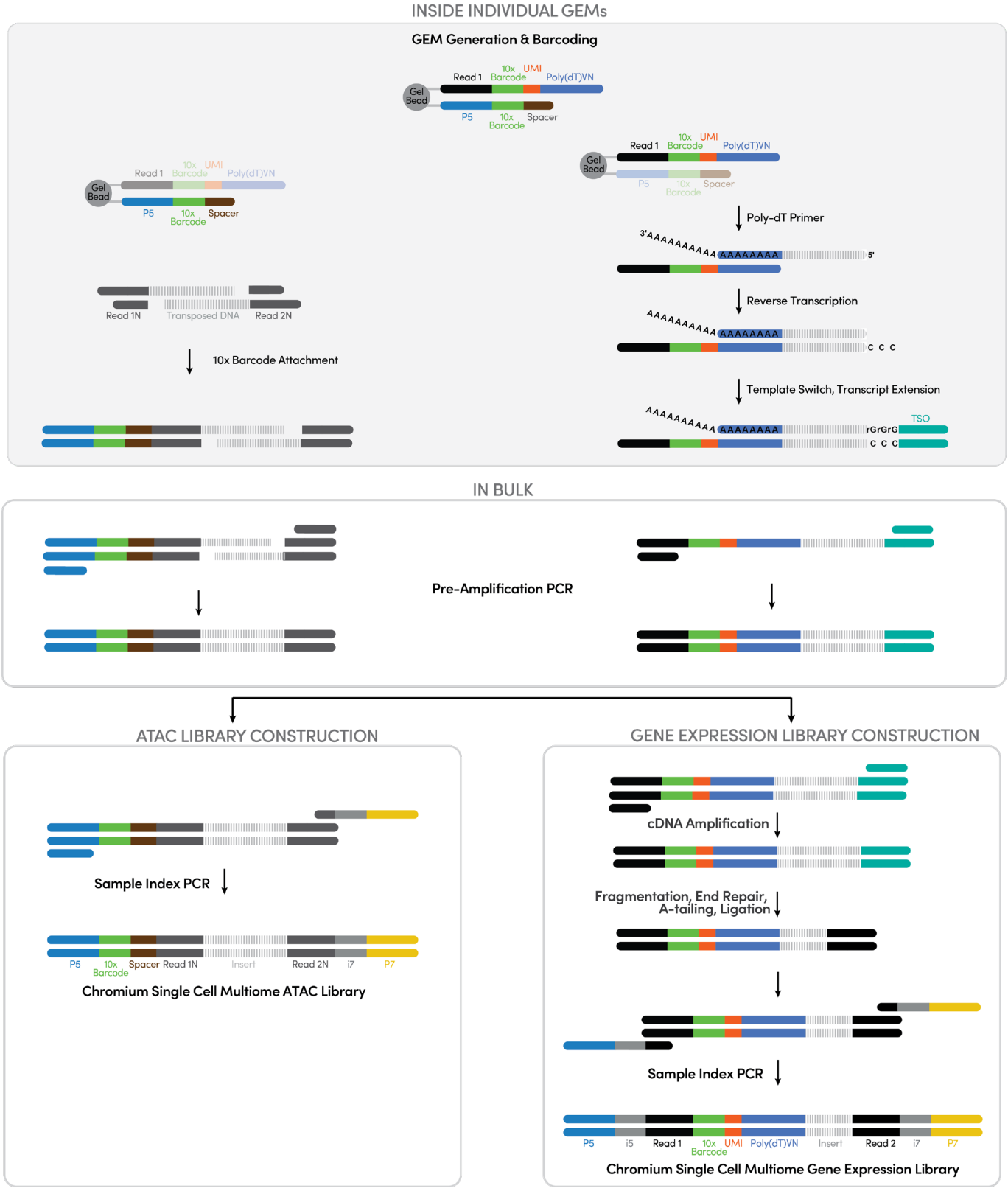
Protocol Step 7.7 – Post Library Construction QC (Gene Expression Library)

Run 10 µl undiluted sample



All traces are representative.

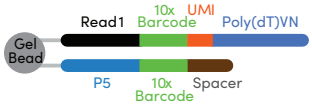
Assay Scheme Overview



Sequences

Single Cell Multiome Gel Beads A (PN- 2000261)

5'-CTACACGACGCTCTTCCGATCT-N16-N12-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-3'

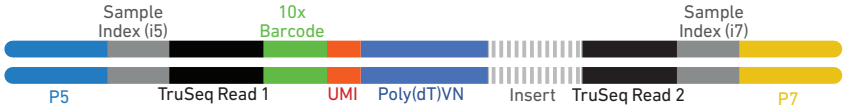


5'-AATGATACGGCGACCACCGAGATCTACAC-N16-CGCGTCTG-3'

Chromium Single Cell Multiome ATAC Library

5'-AATGATACGGCGACCACCGAGATCTACAC-N16-CGCGTCTG-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG---insert---CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-N8-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-GCGCAGAC-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC---insert---GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-N8-TAGAGCATACGGCAGAAGACGAAC-5'

Chromium Single Cell Multiome Gene Expression Library



5'-AATGATACGGCGACCACCGAGATCTACAC-N10-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N12-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN-cDNA_Insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-N10-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-N10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N12-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAABN-cDNA_Insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-N10-TAGAGCATACGGCAGAAGACGAAC-5'