CG000496 Rev C

USER GUIDE

Chromium Next GEM Single Cell ATAC Reagent Kits v2

FOR USE WITH

Chromium Next GEM Single Cell ATAC Kit v2, 16 rxns PN-1000390
Chromium Next GEM Single Cell ATAC Kit v2, 4 rxns PN-1000406
Chromium Next GEM Chip H Single Cell Kit, 48 rxns PN-1000161
Chromium Next GEM Chip H Single Cell Kit, 16 rxns PN-1000162
Single Index Kit N, Set A, 96 rxns PN-1000212

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.

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Document Revision Summary

Document Number	CG000496
Title	Chromium Next GEM Single Cell ATAC Reagent Kits v2 User Guide
Revision	Rev B to C
Revision Date	September 2024

Specific Changes:

- Updated thermal cycler recommendations (page 9)
- Updated to include 10x Magnetic Separator B (pages 9, 19, 34. 39) and Chromium X Series Accessory Kit (page 9)
- Updated glycerol loading volume for row labeled 3, step 2.2a (page 28)
- Updated to include Novaseq™6000 guidance (pages 45-46)

General Changes:

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

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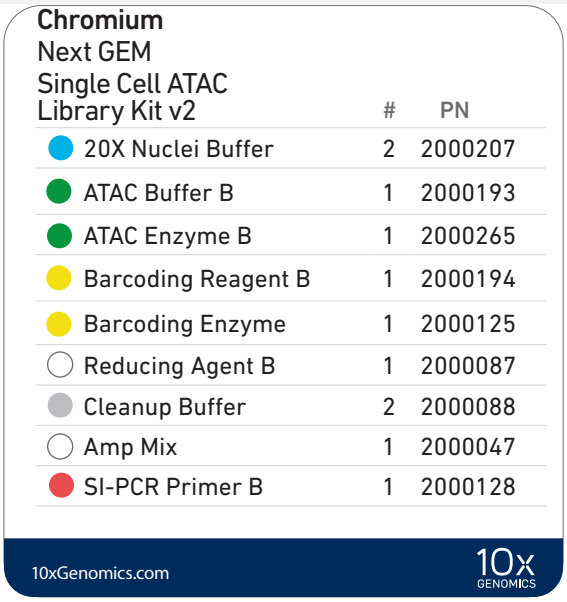
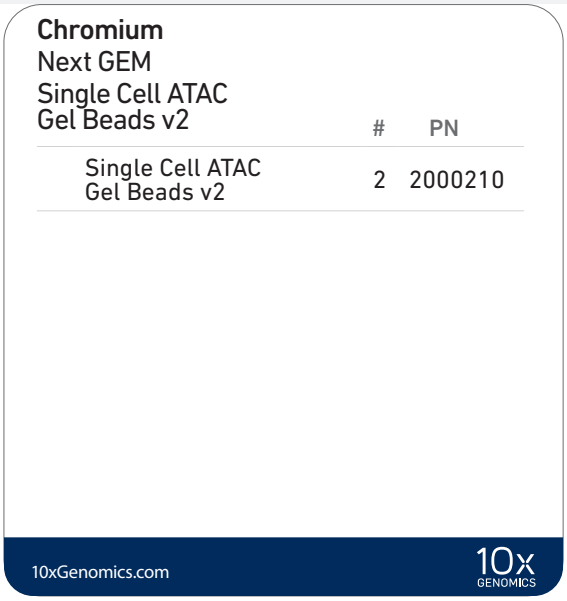
Chromium Next GEM Single Cell ATAC Reagent Kits v2

Chromium Next GEM Single Cell ATAC Kit v2, 16 rxns PN-1000390

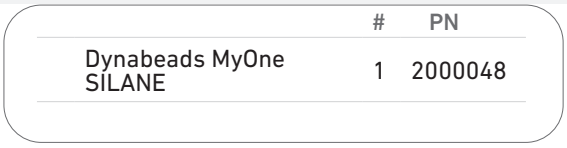
Chromium Next GEM Single Cell ATAC Library Kit v2, 16 rxns PN-1000392 (store at –20°C)



Chromium Next GEM Single Cell ATAC Gel Bead Kit v2, 16 rxns PN-1000391 (store at –80°C)



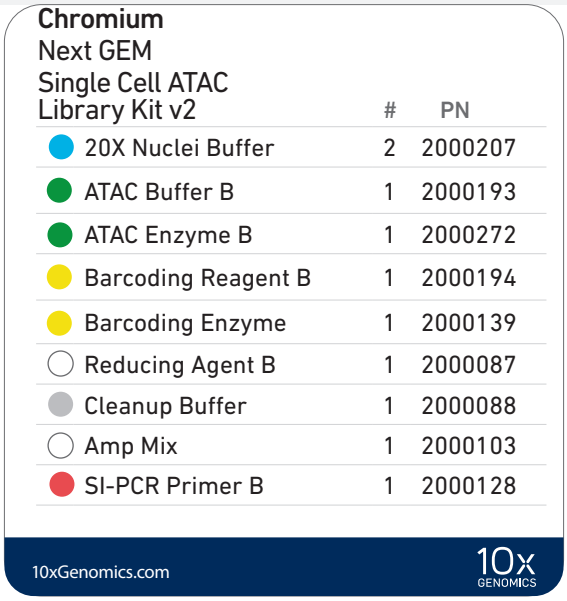
Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)



Chromium Next GEM Single Cell ATAC Reagent Kits v2

Chromium Next GEM Single Cell ATAC Kit v2, 4 rxns PN-1000406

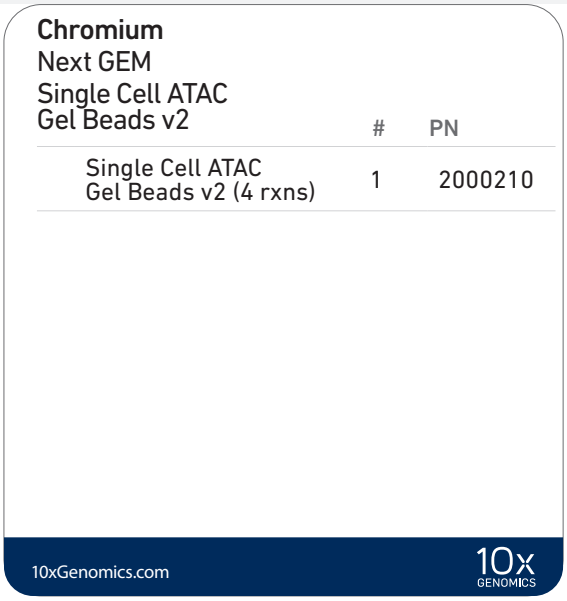
Chromium Next GEM Single Cell ATAC Library Kit v2, 4 rxns PN-1000408 (store at –20°C)



Chromium
Next GEM
Single Cell ATAC
Library Kit v2

	#	PN
20X Nuclei Buffer	2	2000207
ATAC Buffer B	1	2000193
ATAC Enzyme B	1	2000272
Barcoding Reagent B	1	2000194
Barcoding Enzyme	1	2000139
Reducing Agent B	1	2000087
Cleanup Buffer	1	2000088
Amp Mix	1	2000103
SI-PCR Primer B	1	2000128

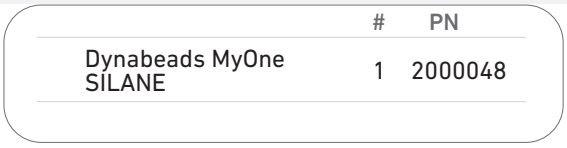
Chromium Next GEM Single Cell ATAC Gel Bead Kit v2, 4 rxns PN-1000407 (store at –80°C)



Chromium
Next GEM
Single Cell ATAC
Gel Beads v2

	#	PN
Single Cell ATAC Gel Beads v2 (4 rxns)	1	2000210

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



	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Chip H Single Cell Kit, 48 rxns PN-1000161 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Next GEM
Chip H & Gaskets

	#	PN
Chromium Next GEM Chip H	6	2000180
Gasket, 6-pack	1	370017

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Chromium Next GEM Chip H Single Cell Kit, 16 rxns PN-1000162 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	2	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	2	220016

Chromium
Next GEM
Chip H & Gaskets

	#	PN
Chromium Next GEM Chip H	2	2000180
Gasket, 2-pack	1	3000072

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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

Chromium Accessories

Product	PN (Orderable)	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 ATAC 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
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
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
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Thermo Fisher Scientific	MYFUGE 12 Mini Centrifuge (alternatively, use any equivalent mini centrifuge)	C1012
Eppendorf	Eppendorf ThermoMixer C Eppendorf ThermoMixer C Bundle, includes SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel (alternatively, use a temperature-controlled Heat Block)	5382000023 2231000574
Quantification & Quality Control		
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	G2943CA 5067-4626 G2991AA 5067-5584 5067-5585
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, or TapeStation 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
2 h	**Nuclei Isolation**			
	Dependent on Cell Type		~1-2 h	
	Step 1 – Transposition			
	1.1	Prepare Transposition Mix	10 min	
	1.2	Isothermal Incubation	30 min	
4 h	**Step 2 – GEM Generation & Barcoding**			
	2.1	Prepare Master Mix	10 min	
	2.2	Load Chromium Next GEM Chip H	10 min	
	2.3	Run the Chromium Controller or X Series	18 min	
	2.4	Transfer GEMs	3 min	
	2.5	GEM Incubation	45 min	STOP 15°C ≤18 h or –20°C ≤ 1 week
6 h	**Step 3 – Post GEM Incubation Cleanup**			
	3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	STOP 4°C ≤ 72 h or –20°C ≤ 2 weeks
	Step 4 – Library Construction			
	4.1	Sample Index PCR	40 min	
	4.2	Post Sample Index Double Sided Size Selection – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	4.3	Post Library Construction QC	60 min	

Stepwise Objectives

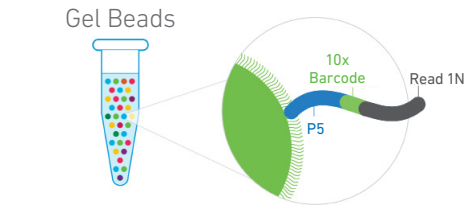
The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. This is achieved by transposing nuclei in a bulk solution; then using a microfluidic chip, the nuclei are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs). A pool of ~750,000 10x Barcodes is sampled to separately and uniquely index the transposed DNA of each individual nucleus. Libraries are generated and sequenced, and 10x Barcodes are used to associate individual reads back to the individual partitions, and thereby, to each individual nucleus.

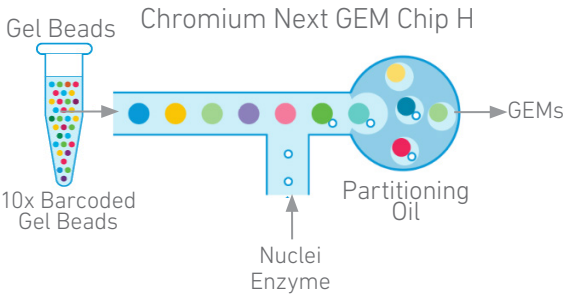
Step 1 Transposition

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.

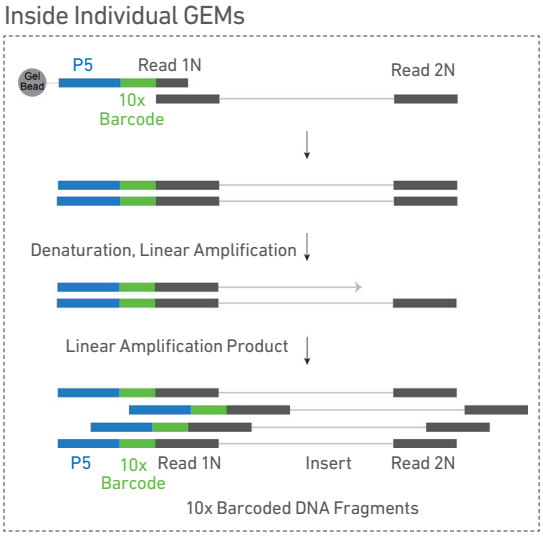
Step 2 GEM Generation & Barcoding

GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Next GEM Chip H. 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 (i) an Illumina® P5 sequence, (ii) a 16 nt 10x Barcode and (iii) a Read 1 (Read 1N) sequence are released and mixed with DNA fragments and Master Mix. Thermal cycling of the GEMs produces 10x barcoded single-stranded DNA. After incubation, the GEMs are broken and pooled fractions are recovered.



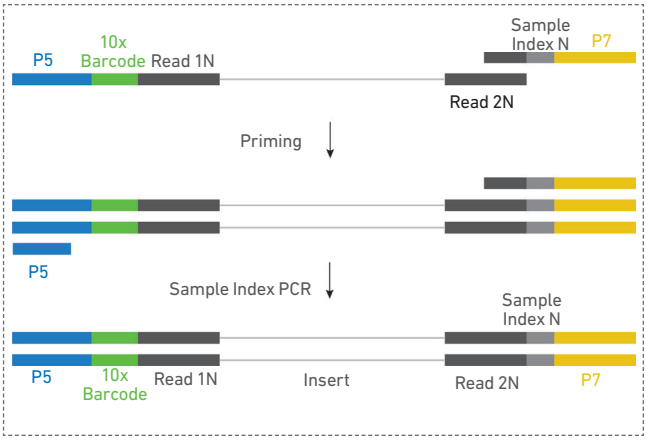
Step 3 Post GEM Incubation Cleanup

Silane magnetic beads are used to remove leftover biochemical reagents from the post GEM reaction mixture. Solid Phase Reversible Immobilization (SPRI) beads are used to eliminate unused barcodes from the sample.

Step 4 Library Construction

P7 and a sample index are added during library construction via PCR. The final libraries contain the P5 and P7 sequences used in Illumina® bridge amplification.

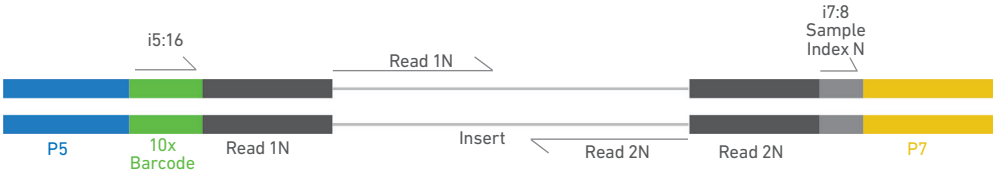
Pooled Amplified DNA Processed in Bulk



Step 5 Sequencing

The Chromium Next GEM Single Cell ATAC Reagent Kits v2 protocol produces Illumina®-ready sequencing libraries. Illumina® sequencer compatibility, sample indices, sequencing depth & run parameters, library loading and pooling are summarized.

Chromium Single Cell ATAC Library



See Appendix for Oligonucleotide Sequences

Tips & Best Practices

Icons

			
Tips & Best Practices section includes additional guidance	Signifies critical step requiring accurate execution	Troubleshooting section includes additional guidance	Version specific protocol step updates

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 1 reaction values.
- 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

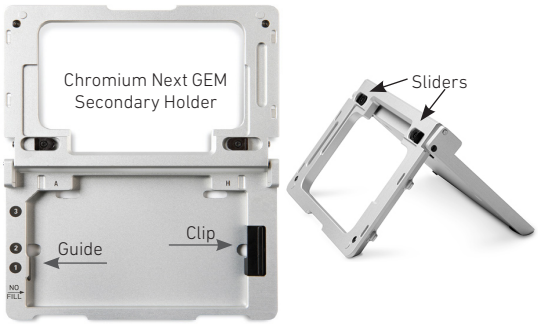
- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

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 within 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 assay.

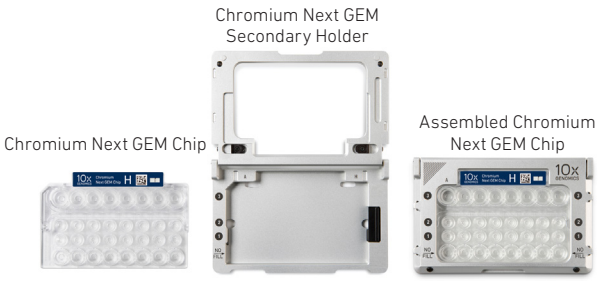
Chromium Next GEM Secondary Holders

- 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.



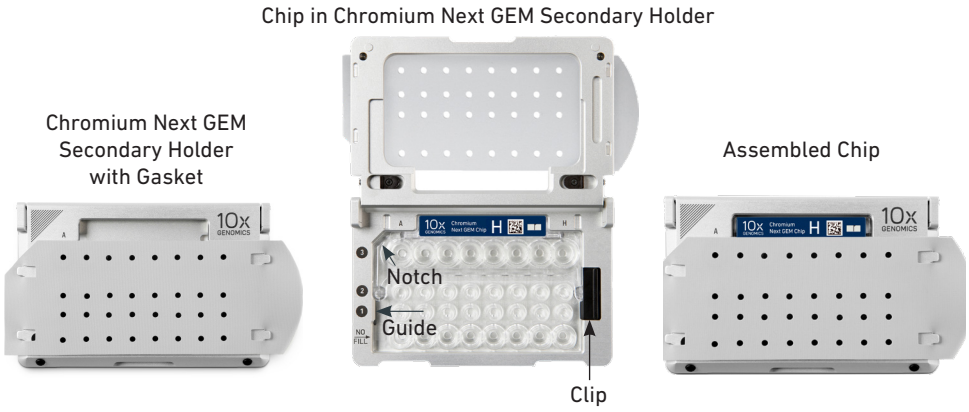
Chromium Next GEM Chip & Holder Assembly

- Align notch on the chip (upper left corner) and the holder.
- Insert the left-hand side of the chip under the guide. Depress the right-hand side of the chip until the spring-loaded clip engages.
- Close the lid before dispensing reagents into the wells.



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.

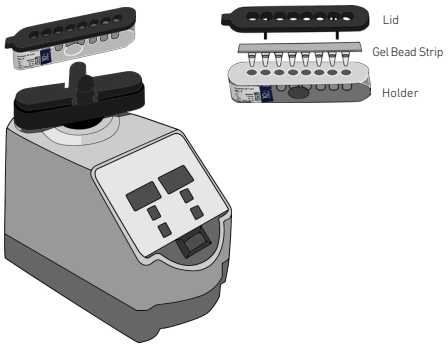


Chromium Next GEM Chip Loading

- Place the assembled chip and holder flat (gasket attached) on the bench with the lid closed.
- 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 H 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.

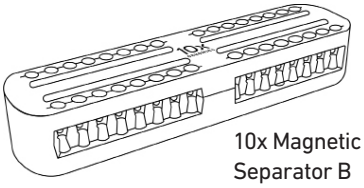


Gel Bead Handling Contd.

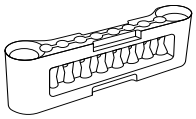
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.
- Centrifuge the Gel Bead strip for ~**5 sec**. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. Place the Gel Bead strip back in the holder and secure the holder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



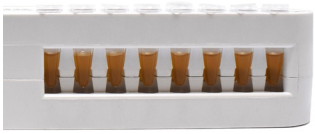
10x Magnetic Separator B



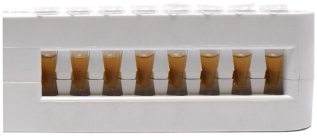
10x Magnetic Separator

Magnetic Bead Cleanup Steps

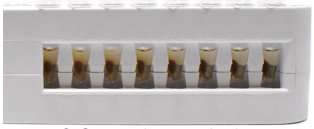
- During magnetic bead based cleanup steps that specify waiting "until the solution clears", visually confirm clearing of solution before proceeding to the next step. See adjacent panel for an example.
- The time need for the solution to clear may vary based on specific step, reagents, volume of reagents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



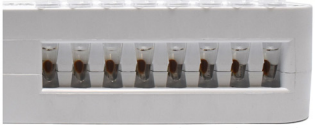
1. Magnetic beads mixed with reagent



2. Separation in progress



3. Separation continuing



4. Separation complete; solution clear

SPRIselect Cleanup & Size Selection

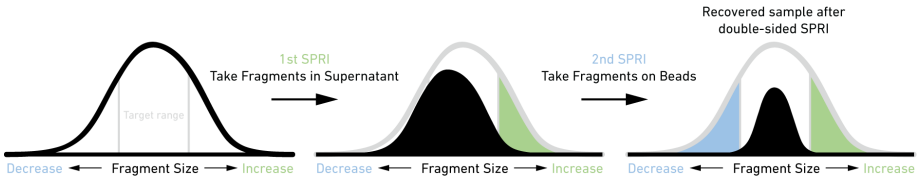
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

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

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 i7 Single Index plate N, Set A contains a unique mix of 4 oligos.
- The sample indexes can therefore be used in any combination.
- Each sample index set is base-balanced to avoid monochromatic signal issues when it is the sole sample loaded on an Illumina® sequencer.

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 isolating nuclei for ATAC Sequencing (Documents CG000169; CG000212). Adhering to this protocol is critical for optimal assay performance. If following a different nuclei isolation protocol, use the Diluted Nuclei Buffer for final nuclei pellet suspension.

⚠ 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
Nuclease-free Water	-	-	950 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	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

Calculate volumes necessary first and proceed to making the Transposition Mix. DO NOT mix Nuclei Stock and Diluted Nuclei Buffer together until indicated in the User Guide (Step 1.1).

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

$$\text{Volume of Nuclei Stock (µl)} = \frac{\text{Targeted Nuclei Recovery x 1.53 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ µl)}}$$

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 1.53

Volume of Nuclei Stock (µl) =

$$\frac{\text{Targeted Nuclei Recovery x 1.53 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/µl)}} = \frac{4000 \times 1.53}{2500} = \mathbf{2.45\ µl}$$

Volume of Diluted Nuclei Buffer = 5 µl - 2.45 µl = **2.55 µl**

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

1.1 Prepare Transposition Mix

Version Specific

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

Version Specific

a. Incubate in a thermal cycler using the following protocol. Ensure lid temperature is set to 50°C. DO NOT use heated lid option if temperature defaults to 100°C.

Lid Temperature	Reaction Volume	Run Time
50°C	15 µl	30 min
Step	**Temperature**	**Time** (hh:mm:ss)
Incubate	37°C	00:30:00
Hold	4°C	Hold

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

Step 2

GEM Generation & Barcoding

2.1 Prepare Reaction Mix

2.2 Load Chromium Next GEM Chip H

2.3 Run the Chromium Controller or X Series

2.4 Transfer GEMs

2.5 GEM Incubation

2.0 GEM Generation & Barcoding

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Single Cell ATAC Gel Beads v2**	2000210	Equilibrate to room temperature 30 min before loading the chip.	–80°C
	○	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
	●	**Barcoding Reagent B**	2000194	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
Place on Ice	●	**Barcoding Enzyme**	2000125/ 2000139	Maintain on ice. Store at –20°C immediately after use.	–20°C
Obtain	●	**Partitioning Oil**	2000190	-	Ambient
		Chromium Next GEM Chip H	2000180	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 4.00 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell ATAC v2 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 B**	2000194	56.5	248.6	497.2
○	**Reducing Agent B**	2000087	1.5	6.6	13.2
●	**Barcoding Enzyme**	2000125/ 2000139	2.0	8.8	17.6
	Total	-	**60.0**	**264.0**	**528.0**

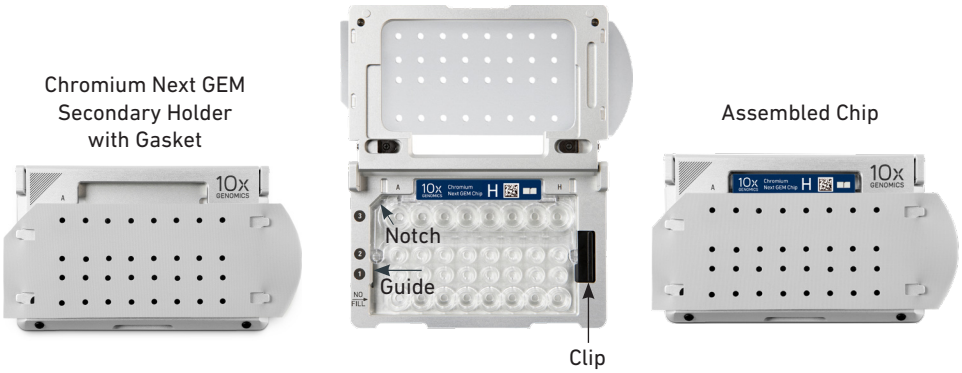
Assemble Chromium Next GEM Chip

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.

Chip in Chromium Next GEM Secondary Holder



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.

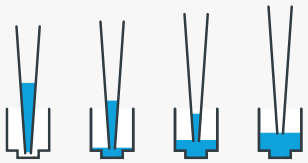
2.2 Load Chromium Next GEM Chip H

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.

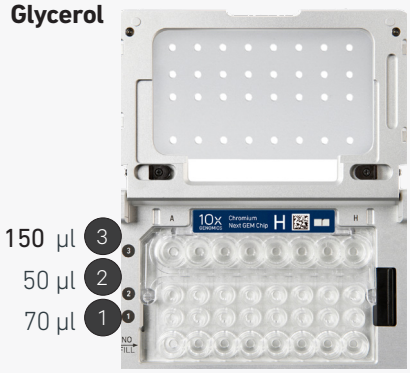


a. Add 50% glycerol solution to each unused well
(if processing <8 samples/chip)

- **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**

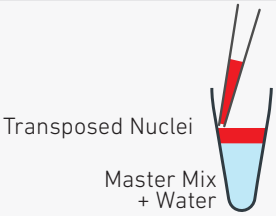
DO NOT add 50% glycerol solution to the bottom row of NO FILL wells.
DO NOT use any substitute for 50% glycerol solution.

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 + Sample

d. Prepare Gel Beads

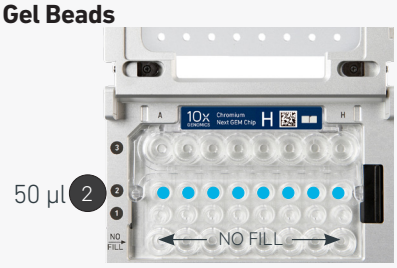
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.**
- Centrifuge the Gel Bead strip for **~5 sec.**
- Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder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

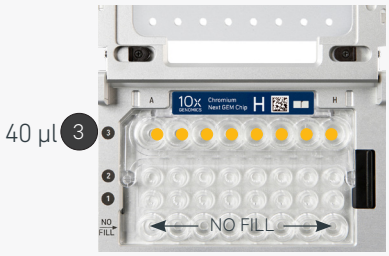


f. Load Row Labeled 3

- Dispense **40 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 Controller.

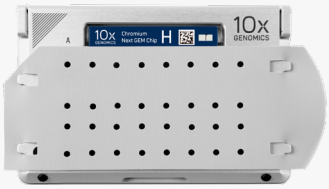
Partitioning Oil



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.



2.3 Run the Chromium Controller or X Series

If using Chromium Controller:

Firmware Version 4.00 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for the Single Cell ATAC v2 protocol.

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

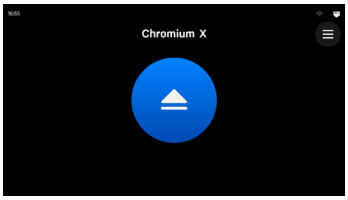
b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Press the play button.

d. At completion of the run **(~18 min)**, the Controller will chime. **Immediately** proceed to the next step.

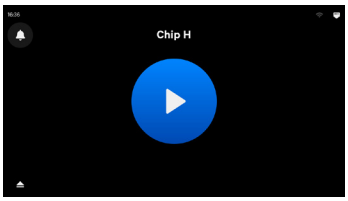
If using Chromium X Series:

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



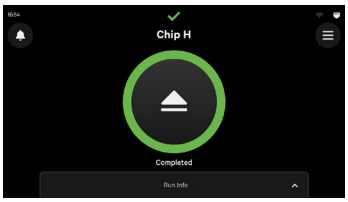
a. Press the eject button on Chromium X Series instrument to eject the tray.
If the eject button is not touched within 1 min, tray will close automatically. System requires a few seconds before the tray can be ejected again.

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 instrumentwill 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.

d. Visually compare the remaining volume in rows labeled 1-2 across all samples. Abnormally high volume in one sample with respect to the others 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.

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.

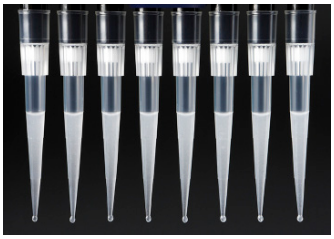
g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the wells.

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**.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



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.

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

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	30 min
Step	**Temperature**	**Time** (hh:mm:ss)
1	72°C	00:05:00
2	98°C	00:00:30
3	98°C	00:00:10
4	59°C	00:00:30
5	72°C	00:01:00 Go to step 3, repeat 11X (Total 12 cycles)
6	15°C	Hold

b. Store at **15°C** for up to **18 h** or at **–20°C** for up to **1 week,** or proceed to the next step.

Step 3

Post GEM Incubation Cleanup

3.0 Post GEM Incubation Cleanup

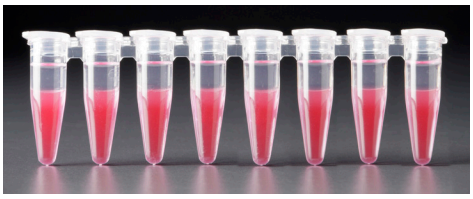
GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	○	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Nuclease-free Water	-	-	-
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥**30 sec**) to resuspend 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, or in a water bath or bead bath, mixing every 5 min. 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	See Tips & Best Practices	Ambient
		Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	-

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).

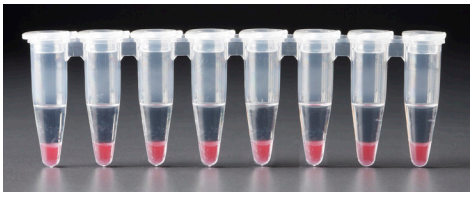
Biphasic Mixture



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

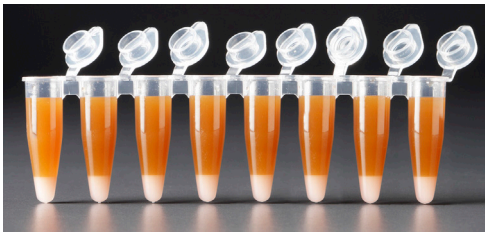


c. Prepare Dynabeads Cleanup Mix.

Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Cleanup Buffer	2000088	182	800.8	1601.6
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	8	35.2	70.4
Reducing Agent B	2000087	5	22	44
Nuclease-free Water	-	5	22	44
Total	-	**200**	**880**	**1760**

Resuspend clump

Add Dynabeads Cleanup Mix



d. Vortex and add **200 µl** to each sample. Pipette mix 5x (pipette set to 200 µl).

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

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

Elution Solution I* *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Buffer EB	-	98.0	431.2	862.4
10% Tween 20	-	1.0	4.4	8.8
Reducing Agent B	200087	1.0	4.4	8.8
Total	-	**100.0**	**440.0**	**880.0**

*Elution Solution I will be used in steps 3.1o and 3.2j

TIPS

g. At the end of **10 min** incubation, place on the 10x Magnetic Separator, high position (magnet•**High**) until the solution clears. A white interface between the aqueous phase and Recovery Agent is normal.

h. Remove the supernatant.

i. Add **300 µl** 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 **40.5 µl** Elution Solution I 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 **40 µl** sample to a new tube strip.

3.2 Post GEM Incubation Cleanup – SPRIselect

a. Vortex the SPRIselect reagent until fully resuspended. Add **48 µl** SPRIselect reagent to each sample. Pipette mix thoroughly using a pipette set to 80 µl.

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.

j. Remove the tube strip from the magnet. **Immediately** add **40.5 µl** Elution Solution I.

k. Pipette mix (pipette set to 30 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 **40 µl** sample to a new tube strip.

o. Store at **4°C** for up to **72 h** or at **–20°C** for up to **2 weeks**, or proceed to the next step.

Step 4

Library Construction

4.0 Library Construction

GET STARTED!				
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Single 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.	-
Place on Ice	**SI-PCR Primer B**	2000128	Vortex, centrifuge briefly.	–20°C
	Amp Mix	2000047/ 2000103	Gently pipette mix, centrifuge briefly.	–20°C
	KAPA Library Quantification Kit for Illumina® Platforms	-	Manufacturer's recommendations.	-
Obtain	**Qiagen Buffer EB**	-	-	Ambient
	10x Magnetic Separator/ 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 Sample Index PCR

Version Specific

Choose the appropriate sample index sets to ensure that no sample indices overlap in a multiplexed sequencing run. Record the 10x Sample Index name used.

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. Add **57.5 µl** Sample Index PCR Mix to **40 µl** sample. Pipette mix and centrifuge briefly.

c. Add **2.5 µl** of an individual Single Index N Set A to each sample and record the well ID used. Pipette mix and centrifuge briefly.

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

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

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

Cycle Number Optimization Table

Targeted Nuclei Recovery	Total Cycles
500-2,000	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.

4.2 Post Sample Index Double Sided Size Selection – SPRIselect

a. Vortex to resuspend SPRIselect reagent. Add **40 µl** SPRIselect reagent to each sample. Pipette mix thoroughly using a pipette set to 130 µl.

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

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

d. Transfer **130 µl** supernatant to a new strip tube.

e. Vortex to resuspend SPRIselect reagent. Add **74 µl** SPRIselect reagent to each sample. Pipette mix thoroughly using a pipette set to 190 µl.

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

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

h. Remove the supernatant. DO NOT discard any beads.

i. With the tube still on 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**.

m. Remove remaining ethanol.

n. Remove from the magnet. **Immediately** add **20.5 µl** Buffer EB. Pipette mix thoroughly using a pipette set to 15 µl.

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

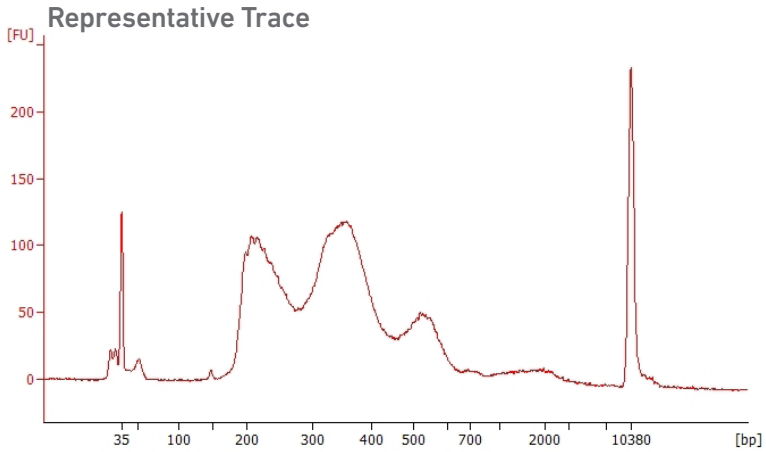
p. Centrifuge briefly. Place on the magnet•**Low** until the solution clears.

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

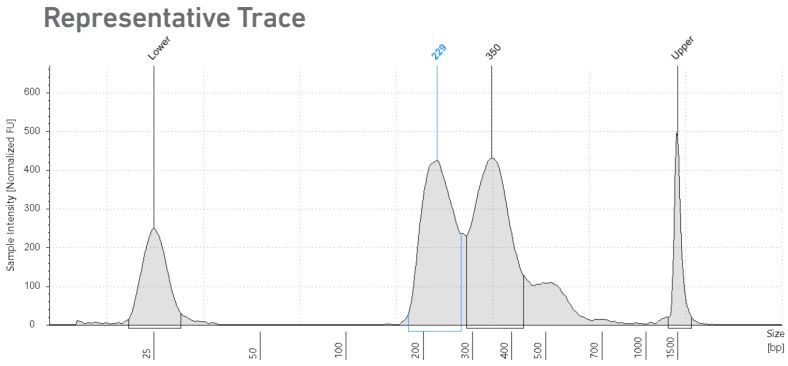
r. Store at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.3 Post Library Construction QC

a. EITHER Run **1 µl** sample at **1:5 dilution** on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size. Lower molecular weight product (≤ 150 bp) and/or a high molecular weight product (~2,000 bp) may be present and is normal. This does not affect sequencing.



b. OR Run **2 µl** sample on the Agilent TapeStation High Sensitivity D1000 or D5000 ScreenTape to determine fragment size.



4.4 Post Library Construction Quantification

a. Thaw KAPA Library Quantification Kit for Illumina® Platforms.

b. Dilute **1 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	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

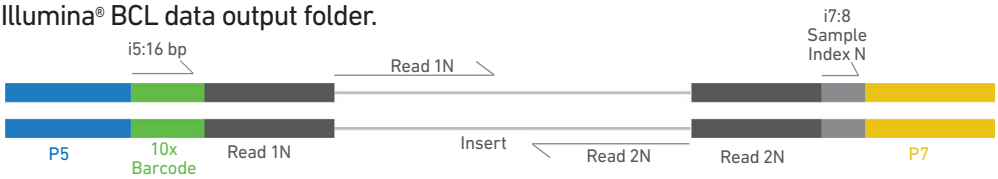
g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using the average size in the region of 175-1,000 bp.

Sequencing

5

Sequencing Libraries

Chromium Single Cell ATAC libraries comprise double stranded DNA fragments which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder.



The BCL data for Single Cell ATAC libraries include:

- Paired-end Read 1N containing insert sequence only
- Read 2N containing insert sequence, starting from the opposite end of fragment
- 8 bp sample index in the i7 read
- 16 bp 10X barcode sequence in the i5 read

The Cell Ranger scATAC pipeline performs demultiplexing and leverages the 10x Barcodes to group read-pairs and associate them to individual cells for secondary analysis and visualization. In addition to performing standard analysis steps such as alignment, Cell Ranger scATAC leverages the 10x Barcodes to generate chromatin accessibility data with single cell resolution. This enables applications including cell clustering, cell type classification, and differential accessibility at a scale of hundreds to thousands of cells.

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 i7 sample index in the Single Index Plate Kit N Set A (PN-3000427) 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 N Set A well ID) is needed in the sample sheet used for generating FASTQs with "cellranger-scATAC mkfastq".

Sequencing Depth & Run Parameters

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	Recommended Number of Cycles
Read 1N	50 cycles
i7 Index	8 cycles
i5 Index	16 cycles
Read 2N	50 cycles

Library Loading

Once quantified and normalized, Single Cell ATAC libraries should be denatured and diluted according to the table below. Consult the Technical Note on Sequencing Metrics and Base Composition of Chromium Single Cell ATAC Libraries (Document CG000181), 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	1.7	1
NextSeq™ 1000/2000	650	1
HiSeq™ 2500 (RR)	11	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*

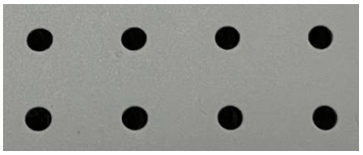
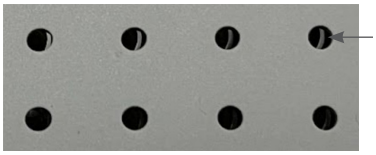
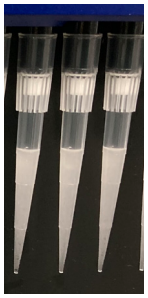
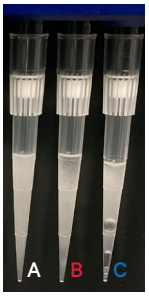
Library Pooling

Pooling dissimilar libraries may compromise the ability to pool effectively due to differences in insert sizes. DO NOT pool Single Cell ATAC libraries with other 10x Genomics libraries.

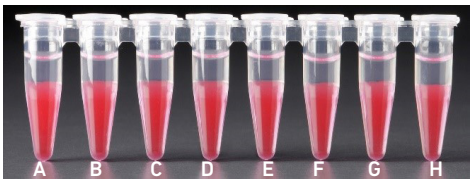
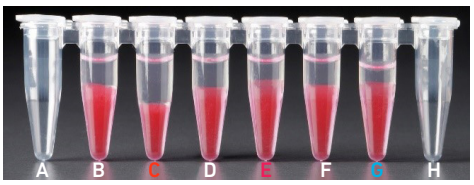
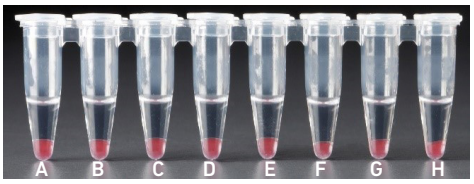
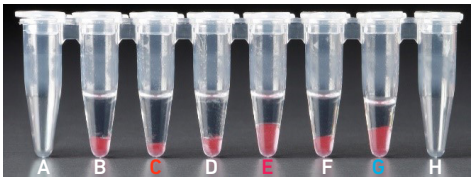
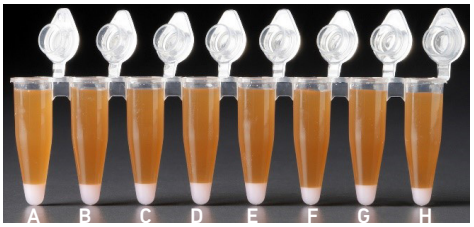
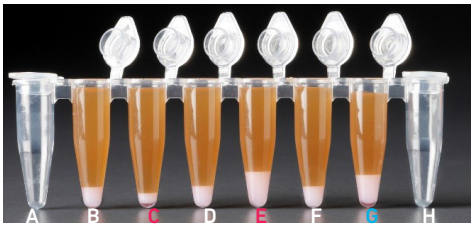
Troubleshooting

6

GEM Generation & Barcoding

STEP	NORMAL	IMPACTED
2.2 Load Chromium Next GEM Chip H	 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 H is removed from the Controller 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 H Recovery Wells	 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, try a new gasket. 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 Series Errors

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

There are two types of errors:

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

a. System Error

b. Pressure Error

c. Chip Error

d. Run Error

e. Temperature Error

f. Software Error

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

a. Gasket Error

b. Tray Error

c. Chip Error

d. Unsupported Chip Error

e. Update Error

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

Appendix

Oligonucleotide Sequences

7

A1 Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell ATAC Reagent Kits v2 User Guide (CG000496)

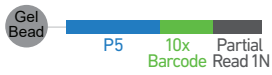
Protocol Step 1 – Transposition

Transposed DNA Product

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----insert-- CTGTCTCTTATACACATCT-3'
3'-TCTACACATATTCTCTGTC --insert-----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5'

Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo Primer
PN-2000210



5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTC-3'

Linear Amplification DNA Product

5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG----insert----CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'

Protocol Step 4.1 – Sample Index PCR

SI-PCR Primer B
PN-2000128

Forward Primer:

5'-AATGATACGGCGACCACCGAGA-3'

Reverse Primer:

5'- CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTCTCGTGGGCTCGG-3'

Single Index Plate N Set A
PN-3000427

Sample Index PCR Product

5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNNNNNNNN-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG---insert---CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'
3'-TTACTATGCCGCTGGTGGCTCTAGATGTG-NNNNNNNNNNNNNNNN-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC---insert---GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-NNNNNNNN-TAGAGCATACGGCAGAAGACGAAC-5'