CG000209 Rev A

USER GUIDE

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

FOR USE WITH

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit, 16 rxns PN-1000175
Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit, 4 rxns PN-1000176
Chromium Next GEM Chip H Single Cell Kit, 48 rxns PN-1000161
Chromium Next GEM Chip H Single Cell Kit, 16 rxns PN-1000162
Chromium i7 Multiplex Kit N, Set A, 96 rxns PN-1000084

Notices

Document Number

CG000209 • Rev A

Legal Notices

© 2019 10X Genomics, Inc. (10x Genomics). All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. A non-exhaustive list of 10x Genomics' marks, many of which are registered in the United States and other countries can be viewed at: www.10xgenomics.com/trademarks. 10x Genomics may refer to the products or services offered by other companies by their brand name or company name solely for clarity, and does not claim any rights in those third-party marks or names. 10x Genomics products may be covered by one or more of the patents as indicated at: www.10xgenomics.com/patents. The use of products described herein is subject to 10x Genomics Terms and Conditions of Sale, available at www.10xgenomics.com/legal-notices, or such other terms that have been agreed to in writing between 10x Genomics and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Notice to Customer: The listed products and their use are the subject of United States Patent Nos. 6,159,736, and 6,294,385, European Patent No. 1115856 and related patents and patent applications licensed from the Wisconsin Alumni Research Foundation.

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

Email: support@10xgenomics.com

10x Genomics

7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA

Document Revision Summary

Document Number	CG000209
Title	Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide
Revision	Rev A
Revision Date	May 2019

- This is Rev A of the Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide.

Table of Contents

Introduction 5
Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 6
Chromium Accessories 9
Recommended Thermal Cyclers 9
Additional Kits, Reagents & Equipment 10
Protocol Steps & Timing 12
Stepwise Objectives 13
Tips &Best Practices 15
Step 1 20
Transposition 21
Nuclei Concentration Guidelines 22
1.1 Prepare Transposition Mix 23
1.2 Isothermal Incubation 23
Step 2 24
GEM Generation & Barcoding 25
2.1 Prepare Master Mix 26
2.2 Load Chromium Next GEM Chip H 27
2.3 Run the Chromium Controller 28
2.4 Transfer GEMs 28
2.5 GEM Incubation 29
Step 3 30
Post GEM Incubation Cleanup 31
3.1 Post GEM Incubation Cleanup – Dynabeads 32
3.2 Post GEM Incubation Cleanup – SPRIselect 34
Step 4 35
Library Construction 36
4.1 Sample Index PCR 37
4.2 Post Sample Index Double Sided Size Selection – SPRIselect 38
4.3 Post Library Construction QC 39
4.4 Post Library Construction Quantification 40
Sequencing 41
Troubleshooting 44
GEMs 45
Chromium Controller Errors 47
Appendix 48

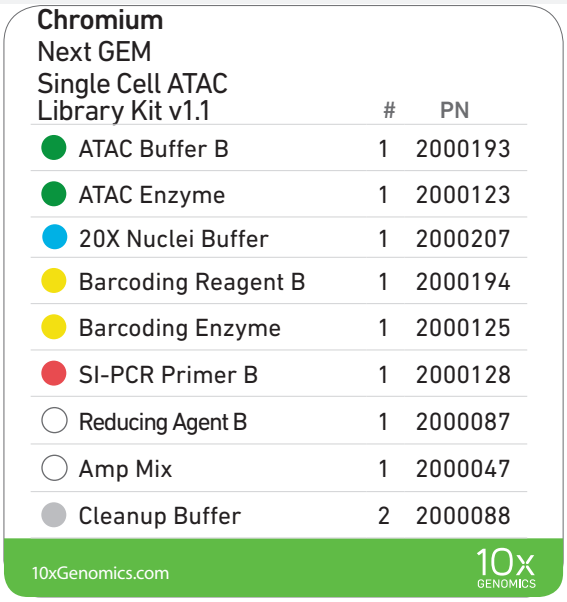
Introduction

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1
Chromium Accessories
Recommended Thermal Cyclers
Additional Kits, Reagents & Equipment
Protocol Steps & Timing
Stepwise Objectives

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1, 16 rxns PN-1000175

Chromium Next GEM Single Cell ATAC Library Kit v1.1, 16 rxns PN-1000163 (store at –20°C)

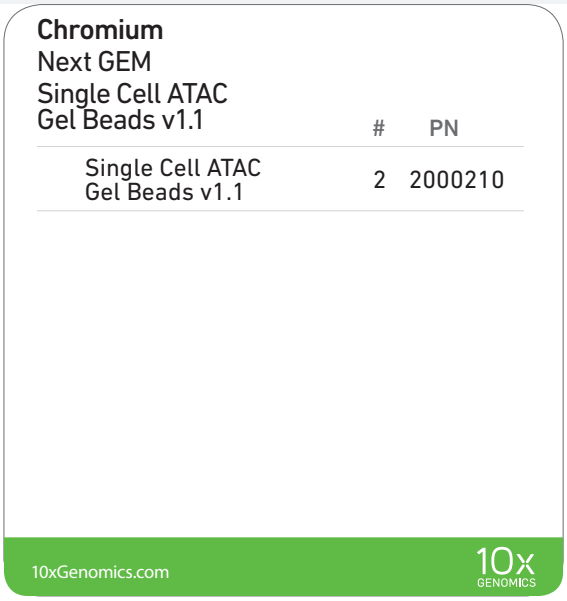


Chromium
Next GEM
Single Cell ATAC
Library Kit v1.1

	#	PN
ATAC Buffer B	1	2000193
ATAC Enzyme	1	2000123
20X Nuclei Buffer	1	2000207
Barcoding Reagent B	1	2000194
Barcoding Enzyme	1	2000125
SI-PCR Primer B	1	2000128
Reducing Agent B	1	2000087
Amp Mix	1	2000047
Cleanup Buffer	2	2000088

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1, 16 rxns PN-1000159 (store at –80°C)

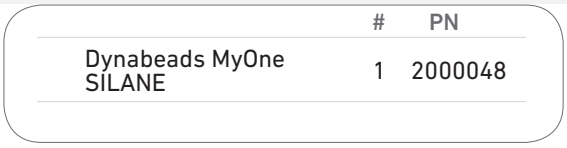


Chromium
Next GEM
Single Cell ATAC
Gel Beads v1.1

	#	PN
Single Cell ATAC Gel Beads v1.1	2	2000210

10xGenomics.com 10x GENOMICS

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



	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Single Cell ATAC Reagent Kits v1.1

Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1, 4 rxns PN-1000176

Chromium Next GEM Single Cell ATAC Library Kit v1.1, 4 rxns PN-1000164 (store at –20°C)

Chromium
Next GEM
Single Cell ATAC
Library Kit v1.1

	#	PN
ATAC Buffer B	1	2000193
ATAC Enzyme	1	2000138
20X Nuclei Buffer	1	2000207
Barcoding Reagent B	1	2000194
Barcoding Enzyme	1	2000139
SI-PCR Primer B	1	2000128
Reducing Agent B	1	2000087
Amp Mix	1	2000103
Cleanup Buffer	1	2000088

10xGenomics.com 10x GENOMICS

Chromium Next GEM Single Cell ATAC Gel Bead Kit v1.1, 4 rxns PN-1000160 (store at –80°C)

Chromium
Next Gem
Single Cell ATAC
Gel Beads v1.1

	#	PN
Single Cell ATAC Gel Beads v1.1 (4 rxns)	1	2000210

10xGenomics.com 10x GENOMICS

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

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Next GEM Chip H Single Cell Kit v1.1, 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

10xGenomics.com

10x GENOMICS

Chromium Next GEM Chip H Single Cell Kit v1.1, 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

10xGenomics.com

10x GENOMICS

Chromium i7 Multiplex Kit N, Set A, 96 rxns PN-1000084 (store at –20°C)

Chromium
i7 Multiplex Kit N
Set A

	#	PN
Chromium i7 Sample Index Plate N, Set A	1	3000262

Chromium Accessories

Product	PN (Orderable)	PN (Item)
10x Vortex Adapter	120251	330002
Chromium Next GEM Secondary Holder	-	3000332
10x Magnetic Separator	120250	230003

Recommended Thermal Cyclers

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

Supplier	Description	Part Number
BioRad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Eppendorf	MasterCycler Pro	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler	4375786

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.

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	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
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
Rainin	Pipet-Lite Multi Pipette L8-50XLS+ Pipet-Lite Multi Pipette L8-200XLS+ Pipet-Lite Multi Pipette L8-10XLS+ Pipet-Lite Multi Pipette L8-20XLS+ Pipet-Lite LTS Pipette L-2XLS+ Pipet-Lite LTS Pipette L-10XLS+ Pipet-Lite LTS Pipette L-20XLS+ Pipet-Lite LTS Pipette L-100XLS+ Pipet-Lite LTS Pipette L-200XLS+ Pipet-Lite LTS Pipette L-1000XLS+	17013804 17013805 17013802 17013803 17014393 17014388 17014392 17014384 17014391 17014382

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

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.

Supplier	Description		Part Number (US)
Quantification & Quality Control			
Agilent	2100 Bioanalyzer Laptop Bundle High Sensitivity DNA Kit 4200 TapeStation High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	Choose Bioanalyzer, or TapeStation based on availability & preference.	G2943CA 5067-4626 G2991AA 5067-5584 5067-5585
KAPA Biosystems	KAPA Library Quantification Kit for Illumina Platforms		KK4824

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	60 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	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	45 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

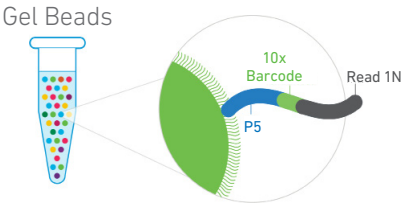
The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. This is achieved by transposing nuclei in a bulk solution; then using a microfluidic chip, the nuclei are partitioned 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.

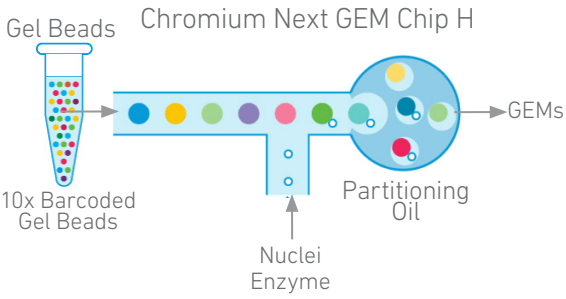
Step 1 Transposition

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.

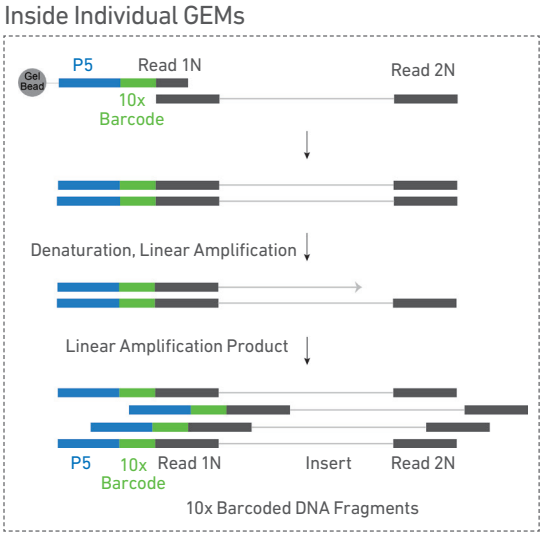
Step 2 GEM Generation & Barcoding

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.

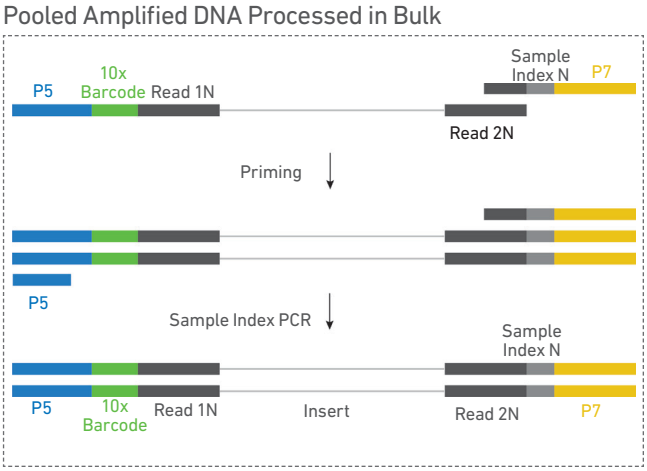


Step 3 Post GEM Incubation Cleanup

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.

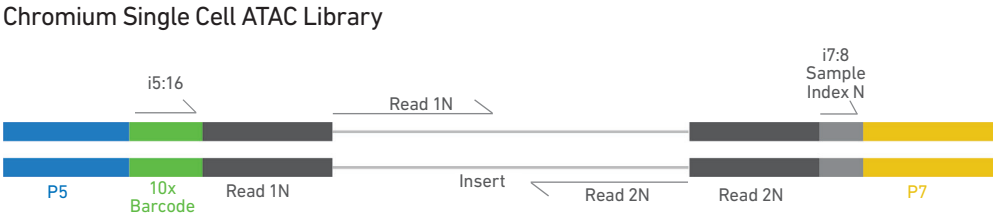
Step 4 Library Construction

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.



Step 5 Sequencing

The Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 protocol produces Illumina®-ready sequencing libraries. Illumina® sequencer compatibility, sample indices, sequencing depth & run parameters, library loading and pooling are summarized.



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	Next GEM 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

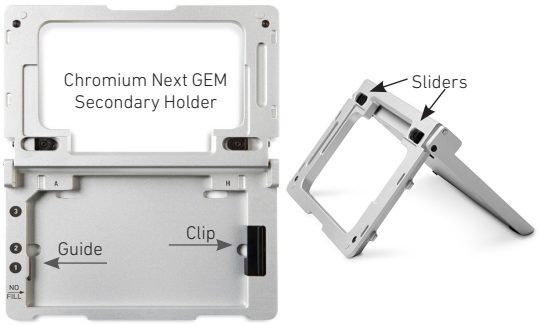
Next GEM

- 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

Next GEM

- 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

Next GEM

- 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 Loading

Next GEM

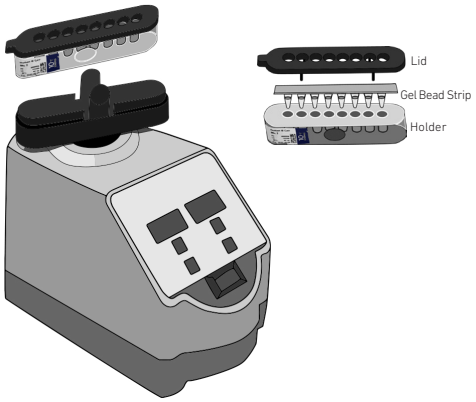
- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Chip H for specific instructions.



Gel Bead Handling

Next GEM

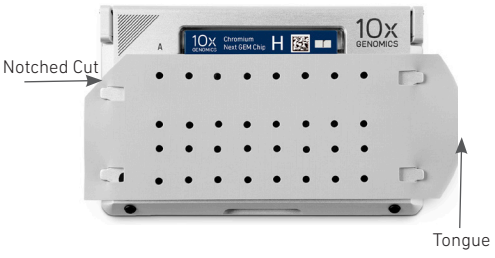
- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.



- 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**. 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.

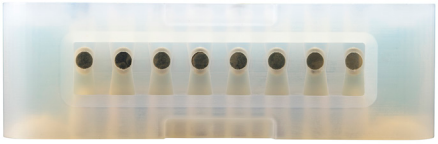
10x Gasket Attachment

- After reagents are loaded, attach the gasket by holding the tongue (curved end, to the right) and hook it 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. DO NOT press down on the top of the gasket after attachment.
- Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.



10x Magnetic Separator

- 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.



SPRIselect Cleanup & Size Selection

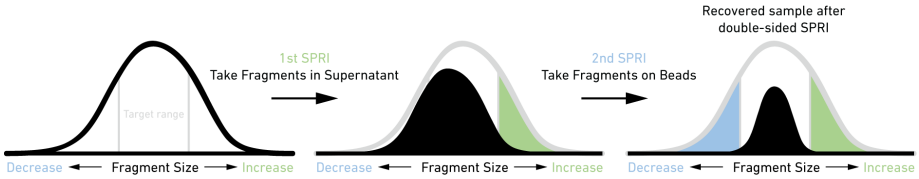
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

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 Sample 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

Next GEM

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**	2000123/ 2000138	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 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

Next GEM

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	2000123/ 2000138	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
50°C	15 µl	60 min
Step	**Temperature**	**Time**
Incubate	37°C	00:60:00
Hold	4°C	Hold

Step 2

GEM Generation & Barcoding

2.1 Prepare Reaction Mix

2.2 Load Chromium Next GEM Chip H

2.3 Run the Chromium Controller

2.4 Transfer GEMs

2.5 GEM Incubation

2.0 GEM Generation & Barcoding

Next GEM

GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature		**Single Cell ATAC Gel Beads v1.1**	2000210	Equilibrate to room temperature 30 min before loading the chip.	–80°C
		Nuclease-free Water	-	-	-
	○	**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 v1.1 protocol.

2.1 Prepare Master Mix

Next GEM

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 H

After removing the chip from the sealed bag, use the chip in ≤ 24 h.

TIPS See Tips & Best Practices for chip handling instructions.



- 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.
- The assembled chip is ready for loading the indicated reagents. Refer to step 2.2 for reagent volumes and loading order.



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

Next GEM

After removing the chip from the sealed bag, use in ≤ 24 h. **For all chip loading steps, raising and depressing the pipette plunger should each take ~5 sec**. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.

a. Dispense 50% Glycerol into Unused Chip Wells (if < 8 samples per chip)

i. **70 µl** to unused wells in **row labeled 1**.
ii. **50 µl** to unused wells in **row labeled 2**.
iii. **40 µl** to unused wells 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.

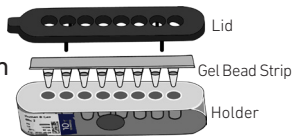
b. Prepare Master Mix + Transposed Nuclei
Add **60 µl** Master Mix to each tube containing Transposed Nuclei for a total of **75 µl** in each tube.

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.



The illustrated chip is being loaded for 8 samples.

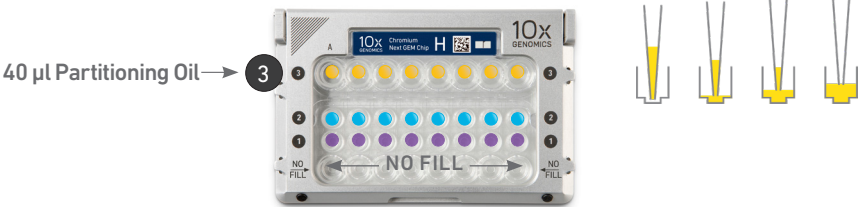
d. Prepare Gel Beads
Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.** Centrifuge the Gel Bead strip for **~5 sec**. Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead strip back in the holder. Secure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



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**.



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.



Attach the gasket and run the chip in the Chromium Controller **immediately** after loading the Partitioning Oil.

g. Attach 10x Gasket
Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.



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

2.3 Run the Chromium Controller

Next GEM

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. Confirm the program on screen. 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 Single Cell ATAC v1.1 protocol.



2.4 Transfer GEMs

Next GEM

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. Check the volume in row labeled 1-2. Abnormally high volume in any well indicates 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**.

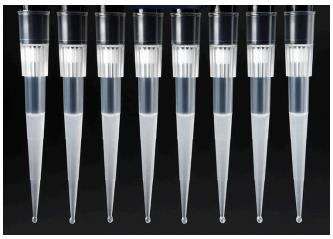
Expose Wells at 45 Degrees



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**
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

3.1 Post GEM Incubation Cleanup – Dynabeads

3.2 Post GEM Incubation Cleanup – SPRIselect

3.0 Post GEM Incubation Cleanup

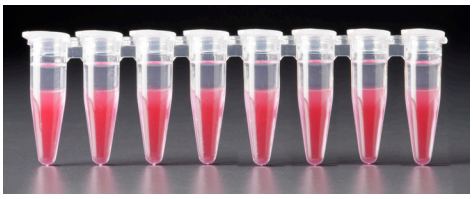
GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	○	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Nuclease-free Water	-	-	-
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥**30 sec**) to resuspend 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	230003	-	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).

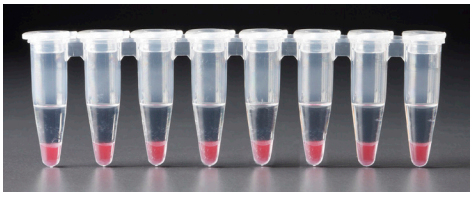
Biphasic Mixture



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

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

e. Incubate **10 min** at **room temperature**.

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 + 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.

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.

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

4.1 Sample Index PCR

4.2 Post Sample Index Double Sided Size Selection – SPRIselect

4.3 Post Library Construction QC

4.4 Post Library Construction Quantification

4.0 Library Construction

GET STARTED!				
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	**Chromium i7 Sample Index Plate N, Set A**	3000262	-	–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	230003	See Tips & Best Practices.	Ambient
	Prepare 80% Ethanol Prepare 10 ml for 8 reactions	-	Prepare fresh.	Ambient

4.1 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. 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 Chromium i7 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
105°C	100 µl	~30 min
Step	**Temperature**	**Time**
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:00:20 Go to step 2, see table below for # cycles
5	72°C	00:01:00
6	4°C	Hold

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

Cycle Number Optimization Table

Targeted Nuclei Recovery	Total Cycles
500-2,000	11
2,001-6,000	10
6,001-10,000	9

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.

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

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



d. Transfer **130 µl** supernatant to a new strip tube. DO NOT discard the supernatant.

e. Vortex to resuspend SPRIselect reagent. Add **74 µl** SPRIselect reagent to each sample. 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 **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.

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

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

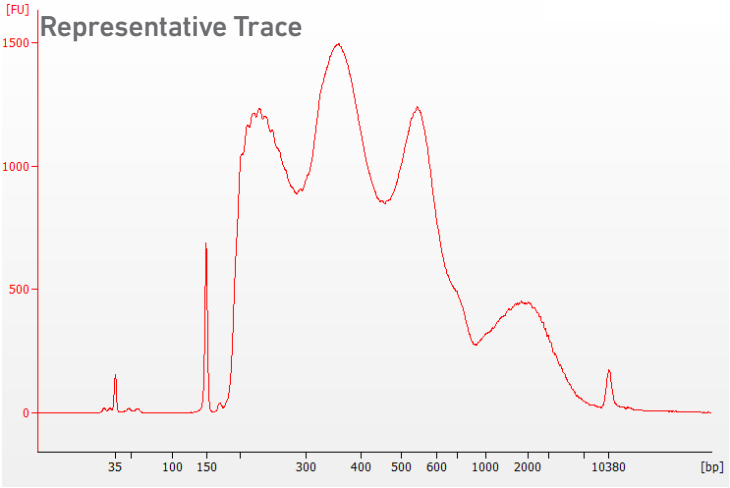
q. Transfer **20 µl** sample to a new tube strip.



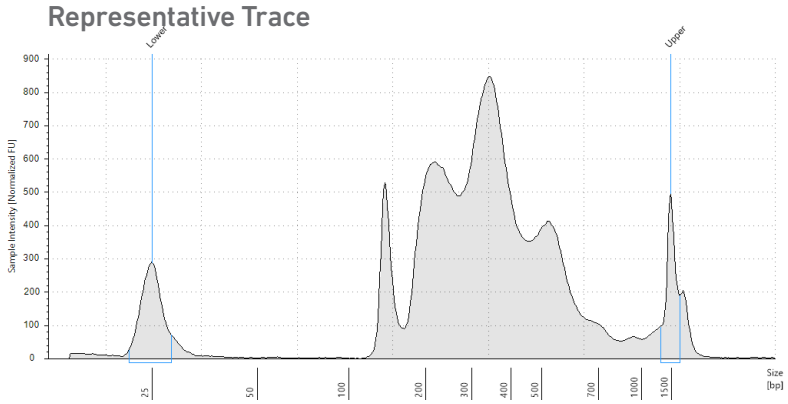
r. Store at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.3 Post Library Construction QC

a. EITHER Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size. Lower molecular weight product (≤ 150 bp) may be present. This does not affect sequencing.



b. OR Run **2 µl** sample on the Agilent TapeStation High Sensitivity D1000 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)	

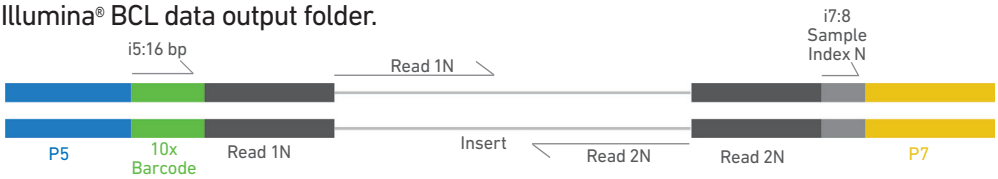
g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using the average size in the region of 175-1,000 bp.

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)
- HiSeq 2500™ (Rapid Run)
- HiSeq™ 3000/4000
- NovaSeq™

Sample Indices

Each sample index in the Chromium i7 Sample Index Plate Kit N, Set A (PN-3000262) 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. the Chromium i7 Sample 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.

Instrument	Loading Concentration (pM)	PhiX (%)
MiSeq™	11	1
NextSeq™ 500/550	1.7	1
HiSeq™ 2500 (RR)	11	1
HiSeq™ 4000	180	1
NovaSeq™	300	1

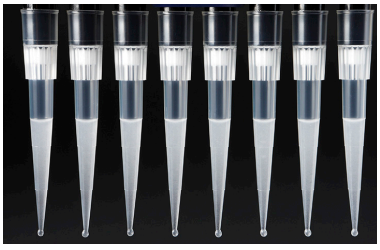
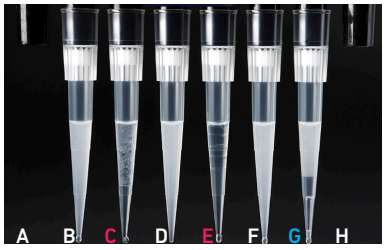
Library Pooling

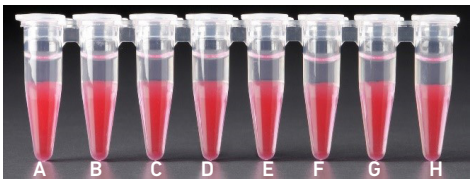
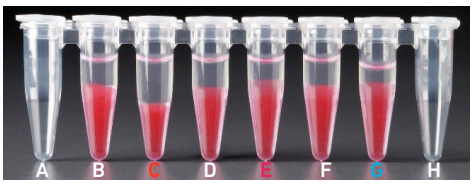
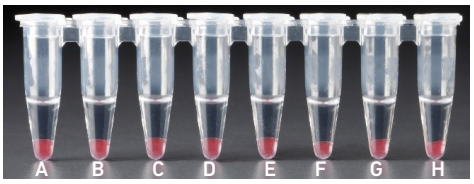
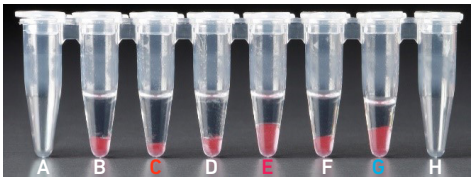
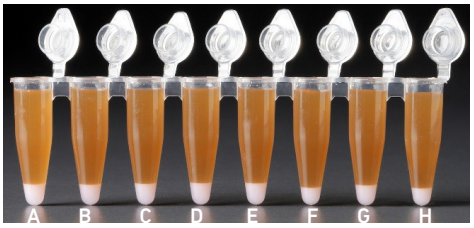
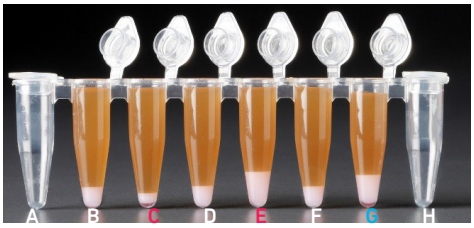
Pooling dissimilar libraries may compromise the ability to pool effectively due to differences in insert sizes. DO NOT pool Single Cell ATAC libraries with other 10x Genomics libraries.

Troubleshooting



GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.4 d After Chip H is removed from the Controller and the wells are exposed	 All 8 recovery wells (row labeled 3) 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 f Transfer GEMs from Chip H Row Labeled 3	All liquid levels are similar in volume and opacity without air trapped in the pipette tips. 	Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog. 

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
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.

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 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. **CAUTION: Chip Holder not Present:** Eject the tray by pressing the eject button to check that the Chromium Next GEM Secondary Holder correctly encases the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

e. **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.

Appendix

Oligonucleotide Sequences

7

Oligonucleotide Sequences

Protocol steps correspond to the Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 User Guide (CG000209)

Protocol Step 1 – Transposition

Transposition Mix

Read 1N primer sequence:
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Read 2N primer sequence:
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

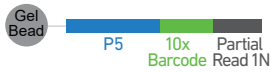
Transposed DNA Product



5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----insert-- CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3'
3'-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC --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'

i7 Sample Index Plate N, Set A PN-3000262

Sample Index PCR Product



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