

CG000168 Rev C

USER GUIDE

Chromium Single Cell ATAC Reagent Kits

FOR USE WITH

Chromium Single Cell ATAC Library & Gel Bead Kit, 16 rxns PN-1000110

Chromium Single Cell ATAC Library & Gel Bead Kit, 4 rxns PN-1000111

Chromium Chip E Single Cell ATAC Kit, 48 rxns PN-1000082

Chromium Chip E Single Cell ATAC Kit, 16 rxns PN-1000086

Chromium i7 Multiplex Kit N, Set A, 96 rxns PN-1000084

Available for use only with the indicated Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263; 120212).

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Notices

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Document Revision Summary

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Specific Changes:

- Updated to specify Chromium Controller compatibility.

General Changes:

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

Table of Contents

Introduction	5
Chromium Single Cell ATAC Reagent Kits	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 Chip E	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
6.1 GEMs	45
6.2 Chromium Controller Errors	47
Appendix	48

Introduction

Chromium Single Cell ATAC Reagent Kits

Chromium Accessories

Recommended Thermal Cyclers

Additional Kits, Reagents & Equipment

Protocol Steps & Timing

Stepwise Objectives

Chromium Single Cell ATAC Reagent Kits

Chromium Single Cell ATAC Library & Gel Bead Kit, 16 rxns PN-1000110

Chromium Single Cell ATAC Library Kit, 16 rxns PN-1000083 (store at -20°C)

Chromium Single Cell ATAC Library Kit		
	#	PN
● ATAC Buffer	1	2000122
● ATAC Enzyme	1	2000123
● Nuclei Buffer	1	2000153
● Barcoding Reagent	1	2000124
● Barcoding Enzyme	1	2000125
● SI-PCR Primer B	1	2000128
○ Reducing Agent B	1	2000087
○ Amp Mix	1	2000047
● Cleanup Buffer	2	2000088

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Chromium Single Cell ATAC Gel Bead Kit, 16 rxns PN-1000081 (store at -80°C)

Chromium Single Cell ATAC Gel Beads		
	#	PN
Single Cell ATAC Gel Beads	2	2000132

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

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Single Cell ATAC Reagent Kits

Chromium Single Cell ATAC Library & Gel Bead Kit, 4 rxns PN-1000111

Chromium Single Cell ATAC Library Kit, 4 rxns PN-1000087 (store at -20°C)

Chromium Single Cell ATAC Library Kit		
	#	PN
● ATAC Buffer	1	2000122
● ATAC Enzyme	1	2000138
● Nuclei Buffer	1	2000153
● Barcoding Reagent	1	2000124
● Barcoding Enzyme	1	2000139
● SI-PCR Primer B	1	2000128
○ Reducing Agent B	1	2000087
○ Amp Mix	1	2000103
● Cleanup Buffer	1	2000088

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Chromium Single Cell ATAC Gel Bead Kit, 4 rxns PN-1000085 (store at -80°C)

Chromium Single Cell ATAC Gel Beads		
	#	PN
Single Cell ATAC Gel Beads (4 rxns)	1	2000132

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

	#	PN
Dynabeads MyOne SILANE	1	2000048

Chromium Chip E Single Cell ATAC Kit, 48 rxns PN-1000082 (store at ambient temperature)

Chromium Partitioning Oil			Chromium Recovery Agent		
	#	PN		#	PN
<input checked="" type="radio"/> Partitioning Oil	6	220088	<input type="radio"/> Recovery Agent	6	220016

Chromium Chip E & Gaskets		
	#	PN
Chip E Single Cell ATAC	6	2000121
Gasket, 6-pack	1	370017

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Chromium Chip E Single Cell ATAC Kit, 16 rxns PN-1000086 (store at ambient temperature)

Chromium Partitioning Oil			Chromium Recovery Agent		
	#	PN		#	PN
<input checked="" type="radio"/> Partitioning Oil	2	220088	<input type="radio"/> Recovery Agent	2	220016

Chromium Chip E & Gaskets		
	#	PN
Chip E Single Cell ATAC	2	2000121
Gasket, 2-pack	1	3000072

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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
10x Chip Holder	120252	330019
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 Chromium Single Cell ATAC protocol. 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	Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.
		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

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Chromium Single Cell ATAC protocol. 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	<div style="border: 1px solid black; padding: 2px; display: inline-block;"> Choose Bioanalyzer, TapeStation or Qubit based on availability & preference. </div> 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 Chip E	10 min	
	2.3 Run the Chromium Controller	7 min	
	2.4 Transfer GEMs	3 min	
	2.5 GEM Incubation	45 min	 15°C ≤ 18 h or -20°C ≤ 1 week
	Step 3 – Post GEM Incubation Cleanup		
	3.1 Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2 Post GEM Incubation Cleanup – SPRIselect	15 min	 4°C ≤ 2 h or -20°C ≤ 2 weeks
6 h	Step 4 – Library Construction		
	4.1 Sample Index PCR	45 min	
	4.2 Post Sample Index Double Sided Size Selection – SPRIselect	20 min	 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). GemCode Technology samples a pool of ~750,000 10x Barcodes 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.

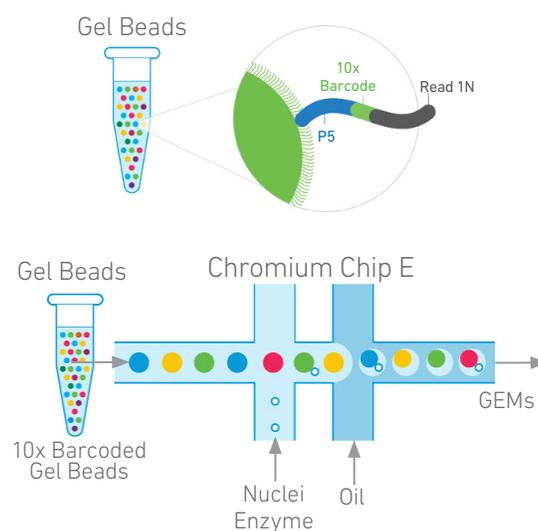
Step 1 Transposition

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.

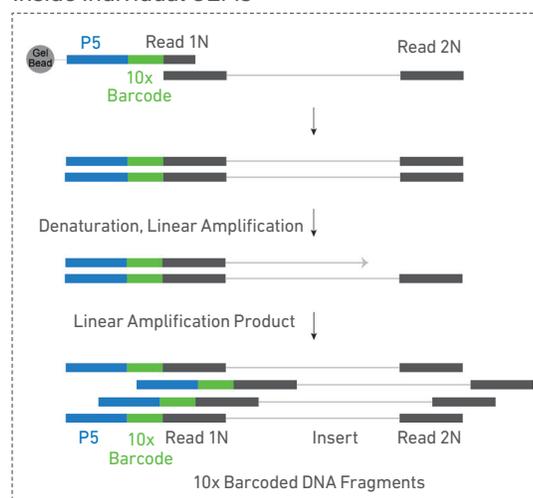
Step 2 GEM Generation & Barcoding

GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Chip E. 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.



Inside Individual GEMs



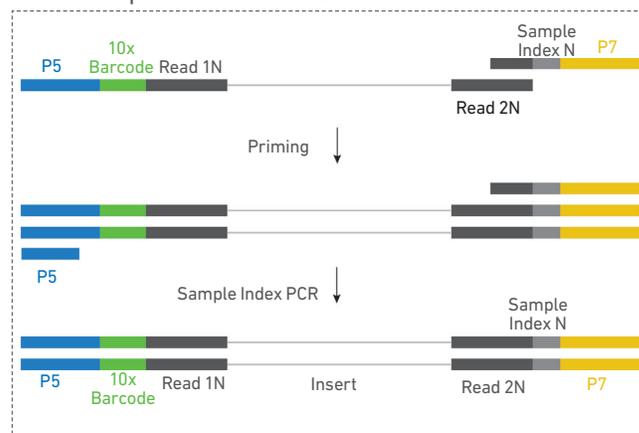
Step 3 Post GEM Incubation Cleanup

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.

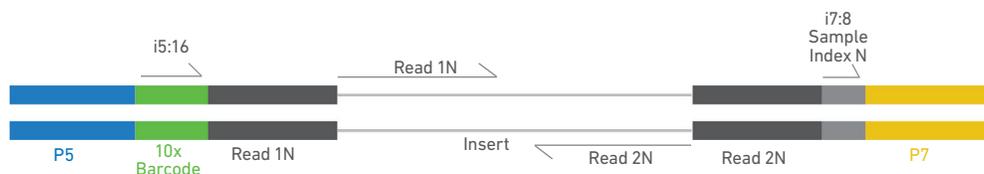
Pooled Amplified DNA Processed in Bulk



Step 5 Sequencing

The Chromium Single Cell ATAC 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

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:
 - Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.
 - Filter through a 0.2- μ m filter.
 - 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 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.
- 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 Recovery Wells.
- 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.

10x Chip Holders

- 10x Chip Holders encase Chromium 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 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 Chip Loading

- Place the assembled chip and holder flat on the bench with the lid closed.
- Dispense at the bottom of the wells without introducing bubbles.
- Wait for the Cell Bead Mix to drain into the bottom of the pipette tips and dispense again to ensure complete volume transfer.
- Refer to [Load Chromium Chip E](#) for specific instructions.



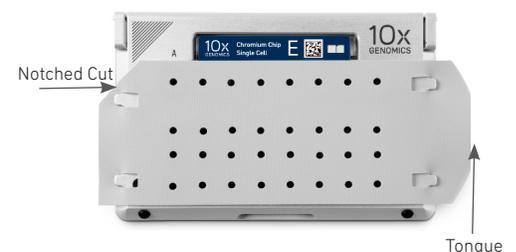
Gel Bead Handling

- 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 .
- Attach a 10x Vortex Adapter to the top of standard laboratory vortexers to vortex the Gel Bead strips.
- After vortexing, remove the Gel Bead strip from the adapter. Flick the Gel Bead strip in a sharp, downward motion to maximize Gel Bead recovery. Confirm there are no bubbles at the bottom of the tubes.
- 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.



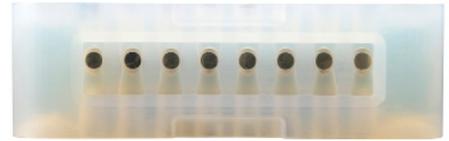
10x Gasket Attachment

- 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

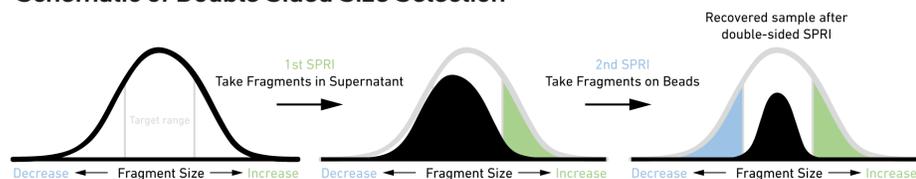
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

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

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

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).

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

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

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

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

1.0 Transposition

GET STARTED!						
Action		Item	10x PN	Preparation & Handling	Storage	
Equilibrate to Room Temperature		ATAC Buffer	2000122	Vortex, centrifuge briefly.	-20°C	
		Nuclei Buffer* *Concentrated 20X stock; dilute 1:20 in nuclease-free water before use. (See below to Prepare Diluted Nuclei Buffer)	2000153	Thaw, Vortex, centrifuge briefly.	-20°C	
Place on Ice		ATAC Enzyme	2000123/ 2000138	Centrifuge briefly.	-20°C	
<p>Nuclei** in Diluted Nuclei Buffer (See below to Prepare Diluted Nuclei Buffer)</p> <p> **Refer to Demonstrated Protocol Nuclei Isolation for ATAC Sequencing (Document CG000169) for isolating nuclei. 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.</p> <p> 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.</p>						
Prepare		Diluted Nuclei Buffer				
			Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
		Nuclei Buffer (PN-2000153)		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

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

$$\text{Volume of Diluted Nuclei Buffer* } (\mu\text{l}) = 5 \mu\text{l} - \text{volume of Nuclei Stock } (\mu\text{l})$$

*Use ONLY Diluted Nuclei Buffer (Dilute Nuclei Buffer (PN-2000153) 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) =

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

$$\text{Volume of Diluted Nuclei Buffer} = 5 \mu\text{l} - 2.45 \mu\text{l} = 2.55 \mu\text{l}$$

Add calculated volumes of Diluted Nuclei Buffer and Nuclei Stock to the Transposition Mix in [step 1.1](#)

1.1 Prepare Transposition Mix

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

Transposition Mix <i>Add reagents in the order listed</i>	PN	1X (μl)	4X + 10% (μl)	8X + 10% (μl)
● ATAC Buffer	2000122	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 Chip E
- 2.3 Run the Chromium Controller
- 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	Chromium Single Cell ATAC Gel Beads	2000132	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	2000124	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	220088	-	Ambient
	Chromium Chip E	2000121	See Tips & Best Practices.	Ambient
	10x Gasket	370017/ 3000072	See Tips & Best Practices.	Ambient
	10x Vortex Adapter	330002	See Tips & Best Practices.	Ambient
	10x Chip Holder	330019	See Tips & Best Practices.	Ambient
	50% glycerol solution If using <8 reactions	-	See Tips & Best Practices.	-



Firmware Version 3.00 or higher is required in the Chromium Controller or the Single Cell Chromium Controller used for the Single Cell ATAC protocol.

2.1 Prepare Master Mix

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

Master Mix <i>Add reagents in the order listed</i>	PN	1X (μl)	4X + 10% (μl)	8X + 10% (μl)
● Barcoding Reagent	2000124	61.5	270.6	541.2
○ Reducing Agent B	2000087	1.5	6.6	13.2
● Barcoding Enzyme	2000125/ 2000139	2.0	8.8	17.6
Total	-	65.0	286.0	572.0

2.2 Load Chromium Chip E

See Tips & Best Practices for chip handling instructions. When loading the chip, raising and depressing the pipette plunger should each take ~5 sec. When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.



a. Assemble Chromium Chip E in a 10x Chip Holder See Tips & Best Practices

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

- i. 75 μ l into unused wells in row labeled 1.
 - ii. 40 μ l into unused wells in row labeled 2.
 - iii. 240 μ l into unused wells in row labeled 3.
- DO NOT add 50% glycerol solution to the top row of Recovery Wells. DO NOT use any substitute for 50% glycerol solution.

c. Prepare Master Mix + Transposed Nuclei

Add 65 μ l Master Mix to each tube containing Transposed Nuclei for a total of 80 μ l in each tube.

d. Load Row Labeled 1

Gently pipette mix the Master Mix + Transposed Nuclei. Using the same pipette tip, dispense 75 μ l Master Mix + Transposed Nuclei into the bottom center of each well in row labeled 1 without introducing bubbles. Wait 30 sec.



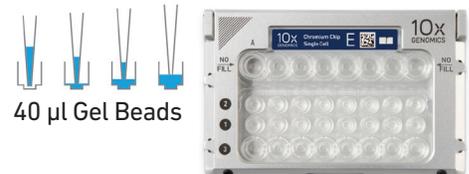
If volume is <75 μ l, load available volume, which should not be <70 μ l.

e. Prepare Gel Beads

Snap the Gel Bead strip into a 10x Vortex Adapter. Vortex 30 sec. Remove the Gel Bead strip and flick in a sharp, downward motion to ensure maximum recovery. Confirm there are no bubbles at the bottom of the tubes and liquid levels look even.

f. Load Gel Beads in Row Labeled 2

Puncture the foil seal of the Gel Bead tubes. Slowly aspirate 40 μ l Gel Beads. Dispense into the bottom of each well in row labeled 2 without introducing bubbles.



g. Load Row Labeled 3

Dispense 240 μ l Partitioning Oil into each well in row labeled 3.



Failure to add Partitioning Oil can damage the Chromium Controller.



h. 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 gasket surface. DO NOT press down on the gasket.

Keep horizontal to avoid wetting the gasket.



2.3 Run the Chromium Controller

- Press the eject button on the Controller to eject the tray.
- Place the assembled chip with the gasket in the tray. Press the button to retract the tray.
- Confirm the program on screen. Press the play button.
- At completion of the run (~7 min), the Controller will chime. **Immediately** proceed to the next step.



2.4 Transfer GEMs

- Place a PCR 8-tube strip on ice.
- Press the eject button of the Controller to remove the chip.
- Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.
- Check the volume in rows 1-3. Abnormally high volume in any well indicates a clog.
- Slowly aspirate **100 μ l** GEMs from the lowest points of the Recovery Wells without creating a seal between the pipette tips and the wells.
- 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.
- Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the wells.
- 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**.



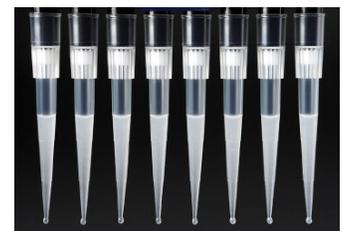
Expose Wells at 45 Degrees



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	<input type="radio"/> 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	<input checked="" type="radio"/> 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 μ l** 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).



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



- b. Slowly remove **125 μ l** Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. **DO NOT** aspirate any aqueous sample.
- c. Prepare Dynabeads Cleanup Mix.

Biphasic Mixture



Remove Recovery Agent



Dynabeads Cleanup Mix <i>Add reagents in the order listed</i>	PN	1X (μ l)	4X + 10% (μ l)	8X + 10% (μ l)
● Cleanup Buffer	2000088	182	800.8	1601.6
Dynabeads MyOne SILANE <i>Vortex thoroughly (\geq30 sec) immediately before adding to the mix.</i>	2000048	8	35.2	70.4
○ Reducing Agent B	2000087	5	22	44
Nuclease-free Water	-	5	22	44
Total	-	200	880	1760



- d. Vortex and add **200 μ l** to each sample. Pipette mix 5x (pipette set to 200 μ l).
- e. Incubate **10 min** at room temperature.

Add Dynabeads Cleanup Mix



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

Elution Solution I <i>Add reagents in the order listed</i>	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
<input type="radio"/> Reducing Agent B	200087	1.0	4.4	8.8
Total	-	100.0	440.0	880.0



- 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** Buffer EB.
- 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	<input checked="" type="radio"/> SI-PCR Primer B	2000128	Vortex, centrifuge briefly.	-20°C
	<input type="radio"/> 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 <i>Add reagents in the order listed</i>	PN	1X (μl)	4X + 10% (μl)	8X + 10% (μl)
<input type="radio"/> Amp Mix	2000047/ 2000103	50	220	440
<input checked="" type="radio"/> 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

Cycle Number Optimization Table

The table recommends a starting point for cycle number optimization for cell lines and primary cells based on Targeted Nuclei Recovery.

Targeted Nuclei Recovery	Cell Lines Total Cycles	Primary Cells Total Cycles
500-2,000	12	13
2,001-6,000	11	12
6,001-10,000	10	11



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



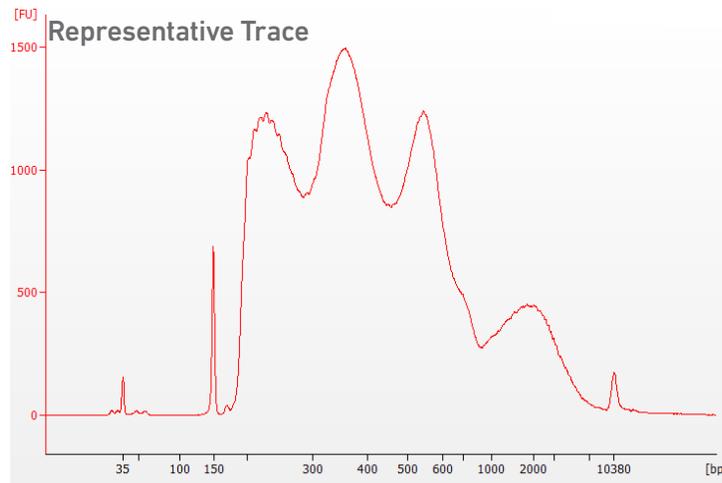
- a. Vortex to resuspend SPRIselect reagent. Add **40 µl** SPRIselect reagent to each sample. Pipette mix.
- b. Incubate **5 min** at **room temperature**.
- c. Place on the magnet•**High** until the solution clears.
- d. Transfer **130 µl** supernatant to a new strip tube. **DO NOT** discard the supernatant.
- 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.



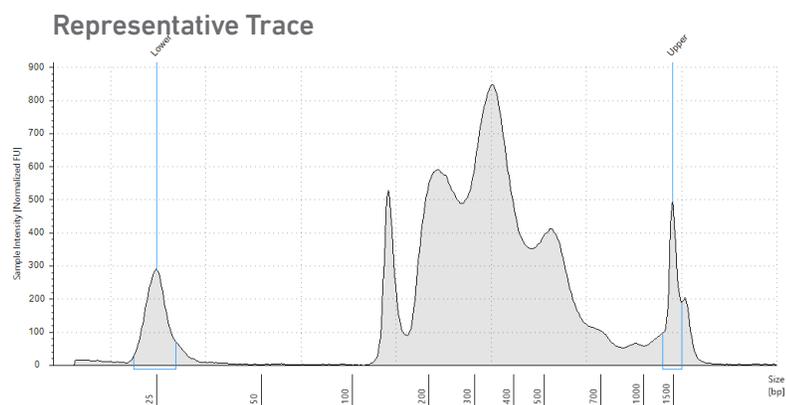
4.3

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)	

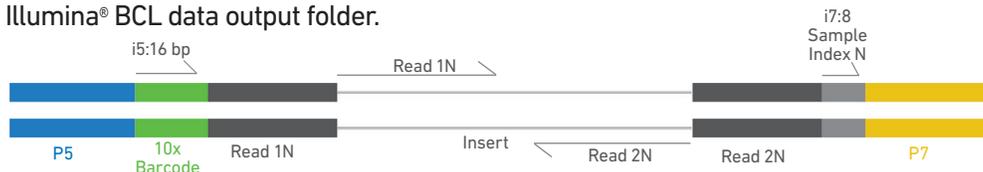
- g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using the average size in the region of 175-1,000 bp.

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	1.7	1
HiSeq™ 2500 (RR)	11	1
HiSeq™ 4000	180	1
NovaSeq™	250	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



6.1 GEMs

STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
------	--------	----------------------------------

2.4 d
After Chip E is removed from the Controller and the wells are exposed



All 8 Recovery Wells are similar in volume and opacity.

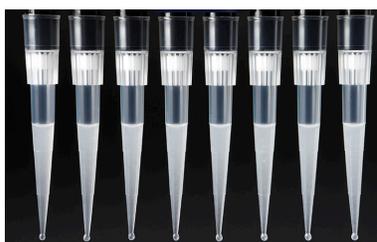


Recovery Well G indicates a reagent clog. Recovery Well C and E indicate a wetting failure. Recovery Wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.

The image indicates clogs in the Gel Bead line (orange arrow) and the sample line (yellow arrow) as evidenced by higher than usual volumes in the input wells.



2.4 e
Transfer GEMs from Chip E Recovery Wells



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.

6.2 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 10x Chip 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 there is a 10x Gasket on the Chromium Chip. In the case when the 10x Gasket installation was forgotten, install and try again. In the case when a 10x Gasket was already installed, remove, reapply, and try again. If the error message is still received after trying either of these more than twice, contact support@10xgenomics.com for further assistance.
- c. **Pressure not at Setpoint:**
 - 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 Chip must be discarded. **Do not try running this Chromium 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 there is a 10x Chip Holder encasing the Chromium Chip. In the case when the 10x Chip Holder was forgotten, install with a 10x Gasket in place, and try again. If the error message is still received after a 10x Chip Holder is confirmed as in place, contact support@10xgenomics.com for further assistance.
- e. **Invalid Chip CRC Value:** This indicates the Chromium Chip has encountered an error, should not be run, and must be discarded. Contact support@10xgenomics.com for further assistance.

Appendix

Oligonucleotide Sequences

7

Oligonucleotide Sequences

Protocol steps correspond to the Chromium Single Cell ATAC Reagent Kits User Guide (CG000168)

Protocol Step 1 – Transposition

Transposition Mix

Read 1N primer sequence:

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'

Read 2N primer sequence:

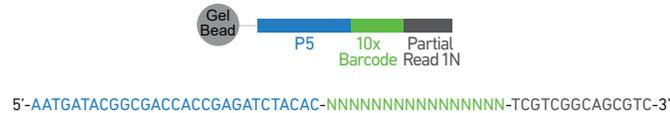
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Transposed DNA
Product



Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo
Primer
PN-2000132



Linear Amplification
DNA Product



Protocol Step 4.1 – Sample Index PCR

SI-PCR Primer B
PN-2000128

Forward Primer:

Partial P5

5'-AATGATACGGCGACCACCGAGA-3'

Reverse Primer:

P7 Sample Index N Partial Read 2N

5'-CAAGCAGAAAGACGGCATAACGAGAT-NNNNNNNNN-GTCTCGTGGGCTCGG-3'

i7 Sample Index
Plate N, Set A
PN-3000262

Sample Index PCR
Product

