CG000168 Rev A

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

Notices

Document Number

CG000168 | Rev A

Legal Notices

Instrument & Licensed Software Updates Warranties

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

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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

10xGenomics.com 10x GENOMICS

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

10xGenomics.com 10x GENOMICS

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

10xGenomics.com 10x GENOMICS

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

Chromium
Single Cell ATAC
Gel Beads

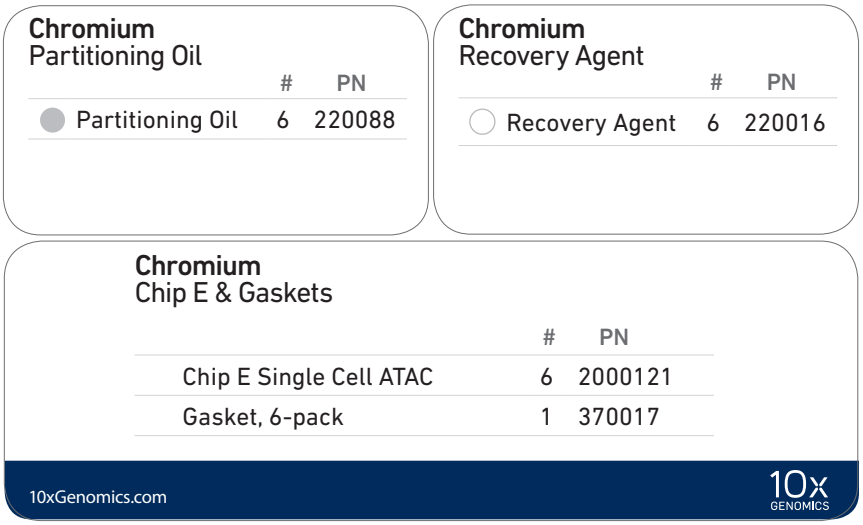
	#	PN
Single Cell ATAC Gel Beads (4 rxns)	1	2000132

10xGenomics.com 10x GENOMICS

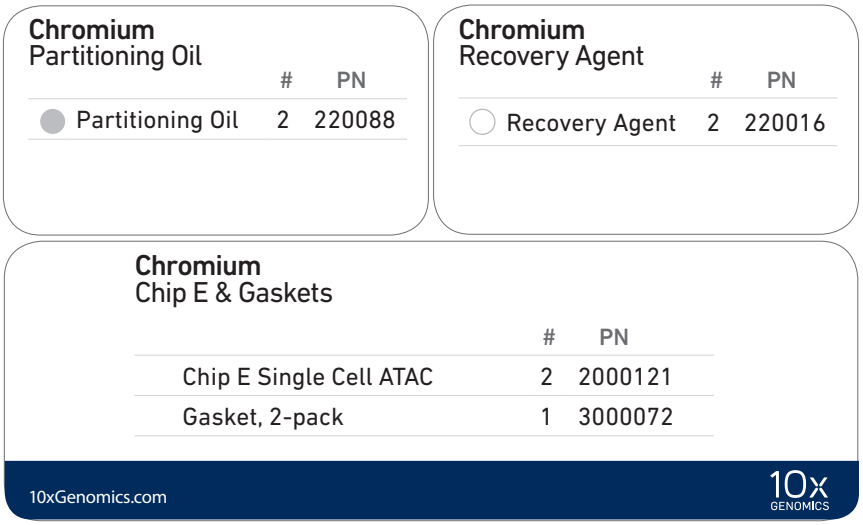
Dynabeads™ MyOne™ SILANE, PN-2000048 (store at 4°C)

	#	PN
Dynabeads MyOne SILANE	1	2000048

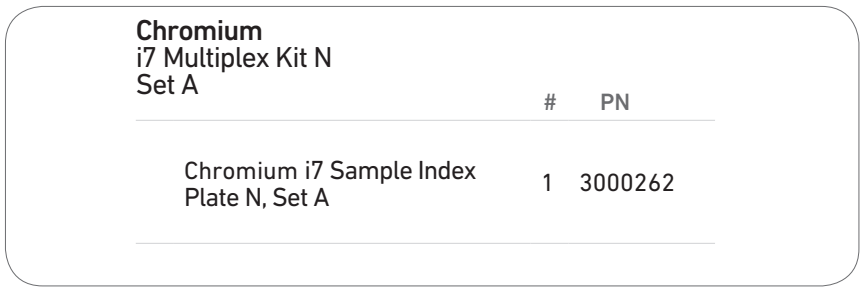
Chromium Chip E Single Cell ATAC Kit, 48 rxns PN-1000082 (store at ambient temperature)



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



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



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 000.01
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 or USA Scientific PCR 8-tube strips.	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip		1402-4700
Rainin	Tips LTS W-O 200UL Filter RT-L200WFLR Tips LTS 20UL Filter RT-L10FLR Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR		30389241 30389226 30389240 30389213
Kits & Reagents			
Thermo Fisher Scientific	Nuclease-free Water		AM9937
Corning Cellgro	Phosphate-Buffered Saline (PBS) 1X without calcium and magnesium		21-040-CV
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)		E7023-500ML
Beckman Coulter	SPRIselect Reagent Kit		B23317
Bio-Rad	10% Tween 20		1662404
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution		3290-32
Qiagen	Qiagen Buffer EB		19086
Equipment			
Rainin	Pipet-Lite Multi Pipette L8-50XLS Pipet-Lite Multi Pipette L8-200XLS		17013804 17013805
VWR	Vortex Mixer Divided Polystyrene Reservoirs		10153-838 41428-958
Eppendorf	ThermoMixer C		5382000015
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 and preferences.	G2943CA 5067-4626 G2991AA 5067-5592 5067-5593
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	
	Step 3 – Post GEM Incubation Cleanup & QC			
	3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	STOP 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	STOP 4°C ≤72 h or –20°C long-term
	4.3	Post Library Construction QC	60 min	

Stepwise Objectives

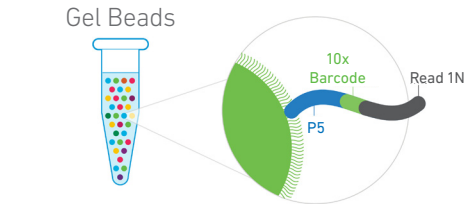
The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. This is achieved by transposing nuclei in a bulk solution; then using a microfluidic chip, the nuclei are partitioned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

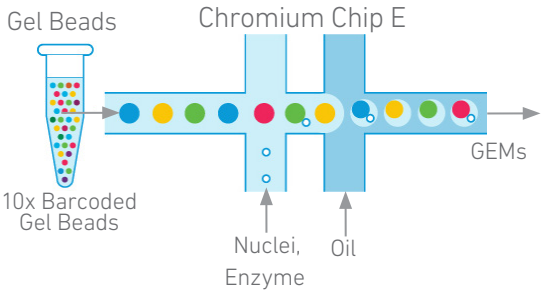
Step 1 Transposition

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

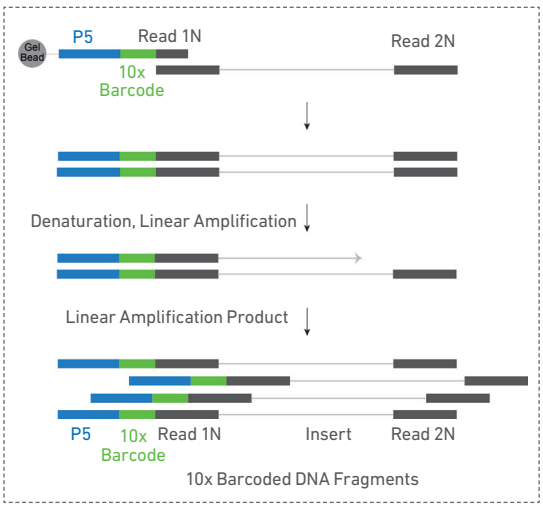
GEMs are generated by combining barcoded Gel Beads, transposed nuclei, a Master Mix, and Partitioning Oil on a Chromium Chip E. To achieve single nuclei resolution, the nuclei are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contains no nuclei, while the remainder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



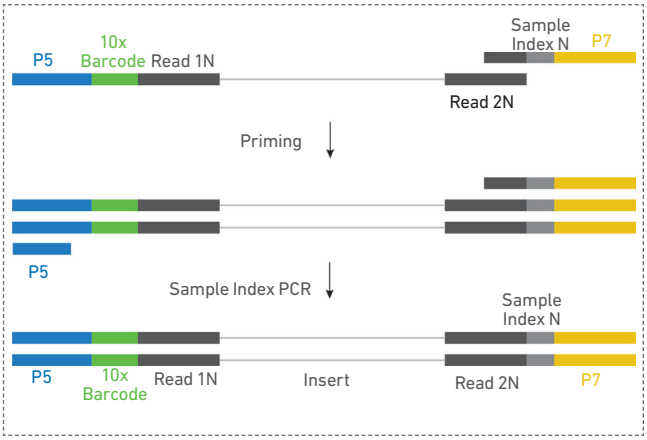
Step 3 Post GEM Incubation Cleanup & QC

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, a sample index, and Read 2 (Read 2N) sequence are added during library construction via PCR. The final libraries contain the P5 and P7 primers used in Illumina® bridge amplification.

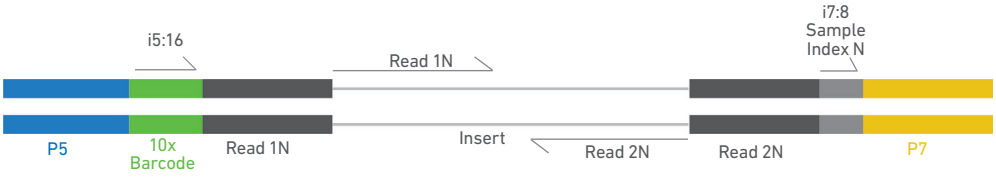
Pooled Amplified DNA Processed in Bulk



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:
  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

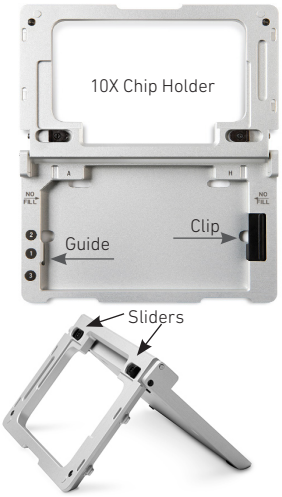
- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

Chromium 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.

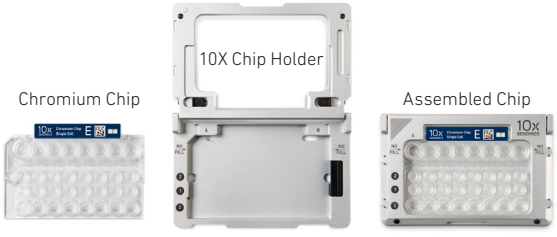
10x Chip Holders

- 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.

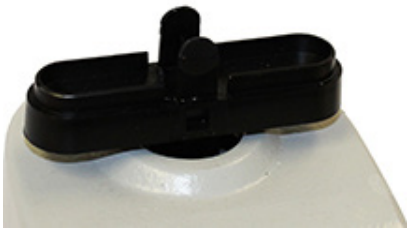


Chromium Chip Loading

- 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

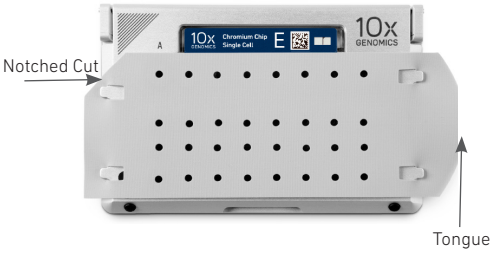
- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Store unused Gel Beads at –80°C and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at –20°C.



- 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.

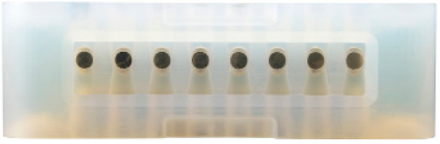
10x Gasket Attachment

- 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

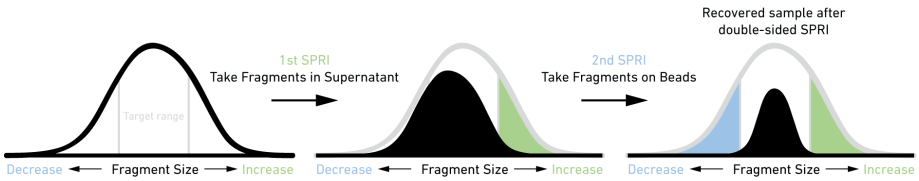
- After aspirating the desired volume of SPRIselect reagent, examine the pipette tips before dispensing to ensure the correct volume is transferred.
- Pipette mix thoroughly as insufficient mixing of sample and SPRIselect reagent will lead to inconsistent results.
- Use fresh preparations of 80% Ethanol.

Tutorial — SPRIselect Reagent:DNA Sample Ratios

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

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

Step 1

Transposition

1.1 Prepare Transposition Mix

1.2 Isothermal Incubation

1.0 Transposition

GET STARTED!

Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	🟢	**ATAC Buffer**	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
		Nuclei** in Diluted Nuclei Buffer (See below to Prepare Diluted Nuclei Buffer)			

⚠ **Refer to **Demonstrated Protocol Nuclei Isolation for ATAC Sequencing (Document CG000167)** 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.

⚠ 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
🔵 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

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

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

*Use ONLY Diluted Nuclei Buffer (Dilute 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) =

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

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

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	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 3.16 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 *Add reagents in the order listed*	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.

TIPS

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)

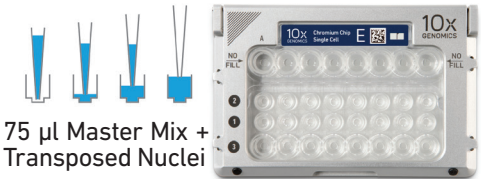
i. **75 µl** into unused wells in **row labeled 1**.
ii. **40 µl** into unused wells in **row labeled 2**.
iii. **240 µl** into unused wells in **row labeled 3**.

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 µl** Master Mix to each tube containing Transposed Nuclei for a total of **80 µl** in each tube.

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

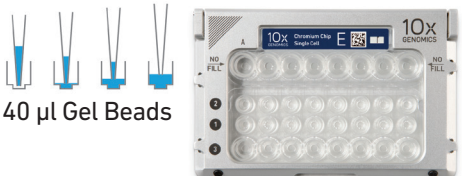
If volume is **<75 µl**, load available volume, which should not be **<70 µl**.



75 µl Master Mix + Transposed Nuclei

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 µl** Gel Beads. Dispense into the bottom of each well in **row labeled 2** without introducing bubbles.



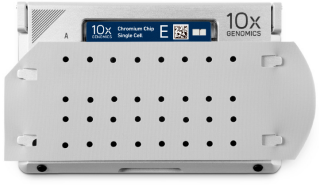
40 µl Gel Beads

g. Load Row Labeled 3
Dispense **240 µl** Partitioning Oil into each well in **row labeled 3**.

Failure to add Partitioning Oil can damage the Chromium Controller.

240 µl Partitioning Oil

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

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

b. Place the assembled chip with the gasket in the tray. Press the button to retract the tray.

c. Confirm the program on screen. Press the play button.

d. At completion of the run **(~7 min)**, the Controller will chime. **Immediately** proceed to the next step.

2.4 Transfer GEMs

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

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

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

Expose Wells at 45 Degrees

d. Check the volume in rows 1-3. Abnormally high volume in any well indicates a clog.

e. Slowly aspirate **100 µl** GEMs from the lowest points of the Recovery Wells without creating a seal between the pipette tips and the wells.

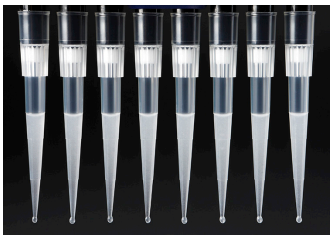
f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

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**.

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	125 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

Step 3

Post GEM Incubation Cleanup & QC

3.0 Post GEM Incubation Cleanup & QC

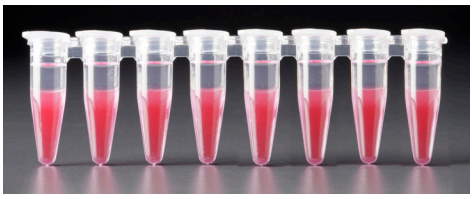
GET STARTED!					
Action		Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature	○	**Reducing Agent B**	2000087	Thaw, vortex, verify no precipitate, centrifuge briefly.	–20°C
		Nuclease-free Water	-	-	-
		Dynabeads MyOne SILANE	2000048	Vortex thoroughly (≥**30 sec**) to resuspend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

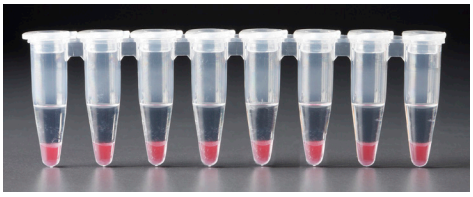
Biphasic Mixture



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

b. Slowly remove **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.	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 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**

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** 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

4.0 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	Vortex, 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 for cell lines and primary cells based on Targeted Nuclei Recovery.

Cycle Number Optimization Table

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

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

TIPS

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.

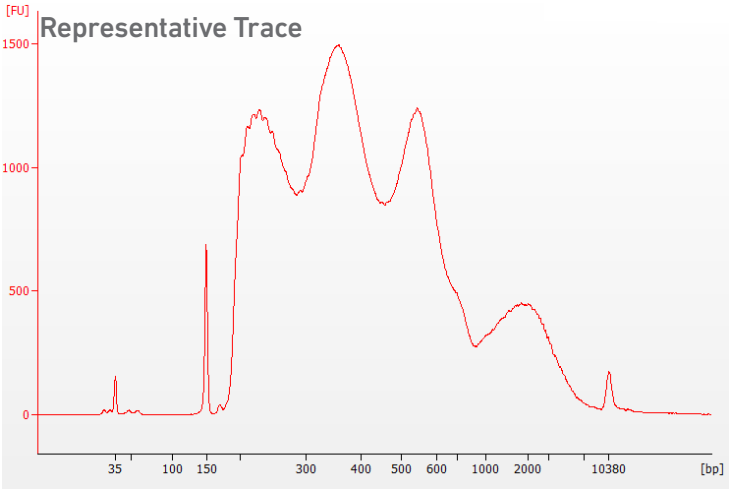
q. Transfer **20 µl** sample to a new tube strip.

STOP

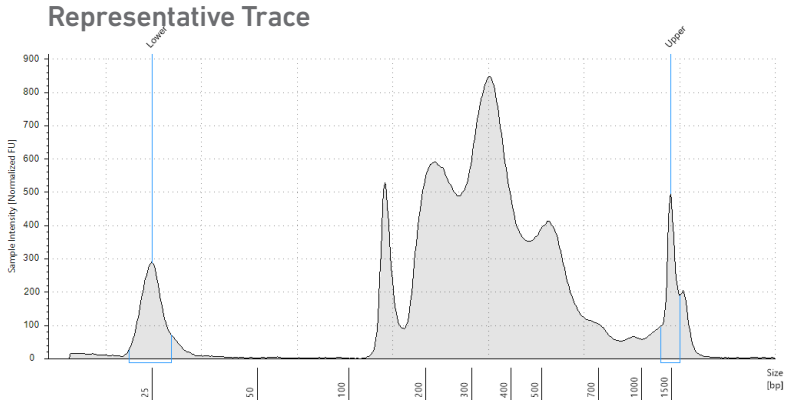
r. Store at **4°C** for up to **72 h** or at **–20°C** for **long-term** storage.

4.3 Post Library Construction QC

a. EITHER Run **1 µl** sample on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size.



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

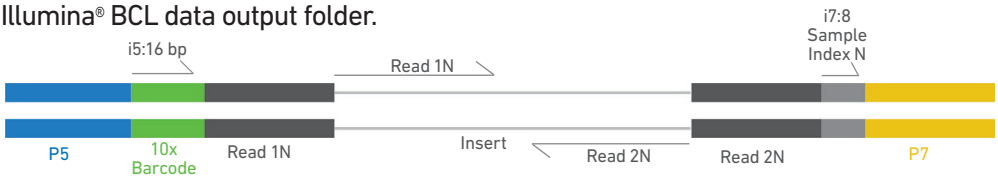
g. Follow the manufacturer's recommendations for qPCR-based quantification. For library quantification for sequencer clustering, determine the concentration using the average size in the region of 150-1000 bp.

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-dna 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

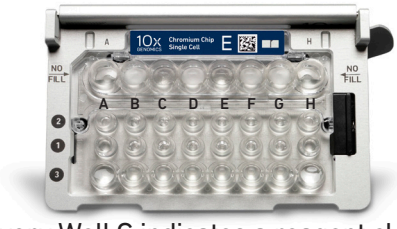
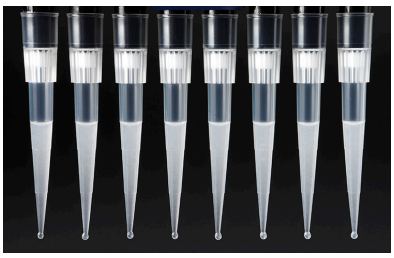
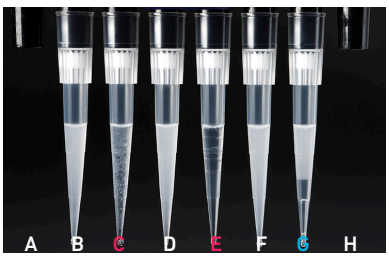
Library Pooling

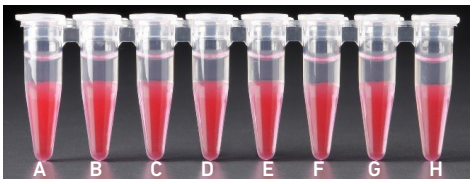
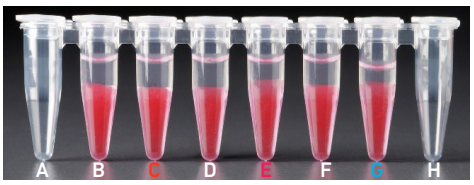
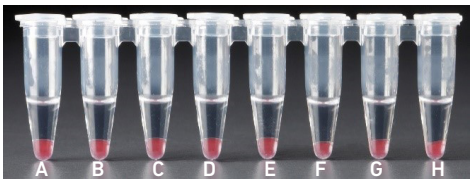
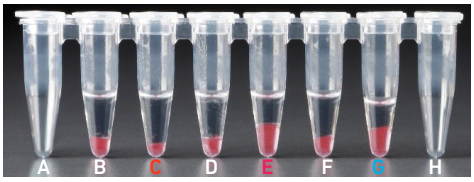
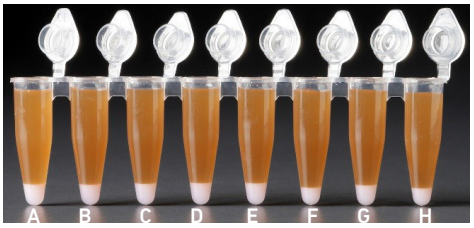
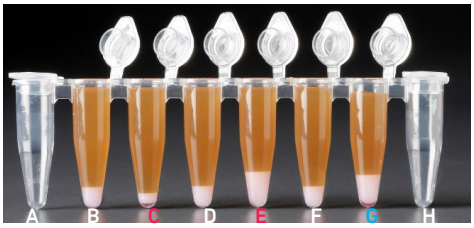
Pooling dissimilar libraries may compromise the ability to pool effectively due to differences in insert sizes. DO NOT pool Single Cell ATAC libraries with other 10x Genomics libraries.

Troubleshooting

6

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.

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 residual 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.

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

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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'

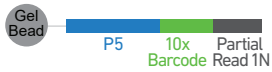
Read 2N primer sequence:
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Transposed DNA Product

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

Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo Primer PN-2000132



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'