scATAC Pre-Indexing Kit

(24-Plex; 110001)

User Guide (10X Chromium Next GEM)

Table of Contents

Introduction

The ScaleBio scATAC Pre-Indexing Kit provides a complementary system to increase sample and cell throughput for on-market single cell analysis systems. When used in combination with the Chromium Next GEM Single Cell ATAC Reagent Kit (1000175/1000176) the scATAC Pre-Indexing Kit can enable users to pool up to 24 samples and load up to 100,000 nuclei per channel of 10X Chromium while maintaining a low effective doublet rate. This is accomplished by indexing samples upstream of the 10X workflow using indexed tagmentation, followed by super loading the pooled and indexed nuclei onto the 10X Genomics Chromium platform, then purifying and sequencing libraries in accordance with the Chromium Next GEM Single Cell ATAC manual (Figure 1). Importantly, in addition to sample identification the Scale indexed tagmentation allows users to distinguish molecules from two or more indexed nuclei within the same droplet, allowing users to superload each 10X Chromium channel and producing a final output of up to 300,000 nuclei from one Scale Pre-Indexing Kit.

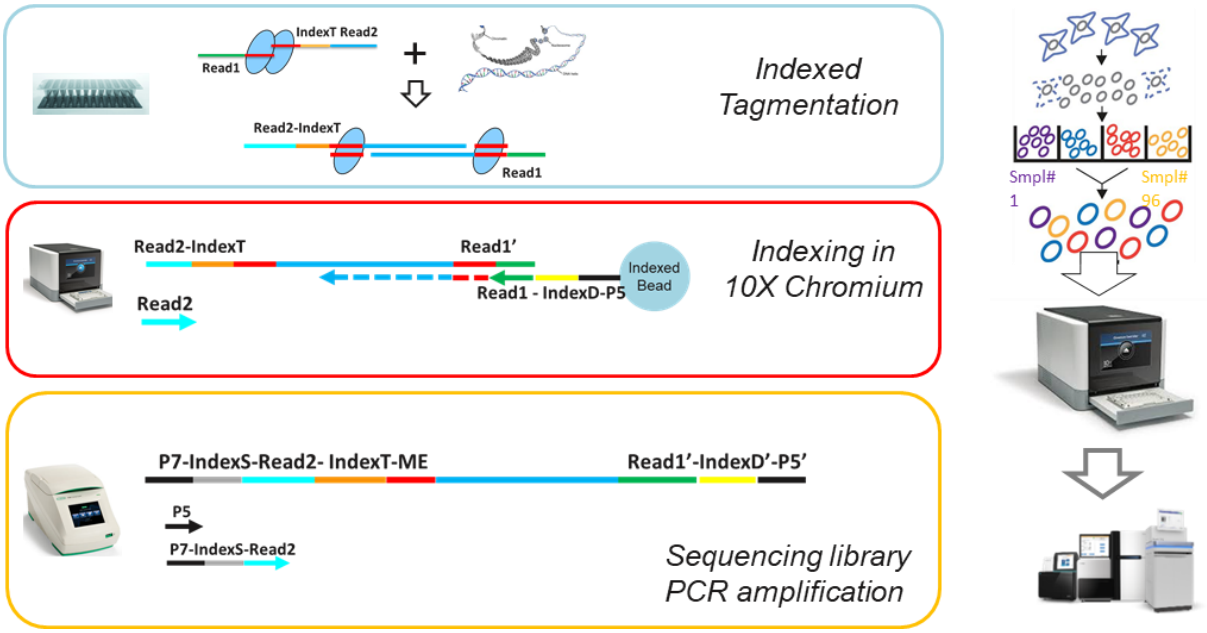
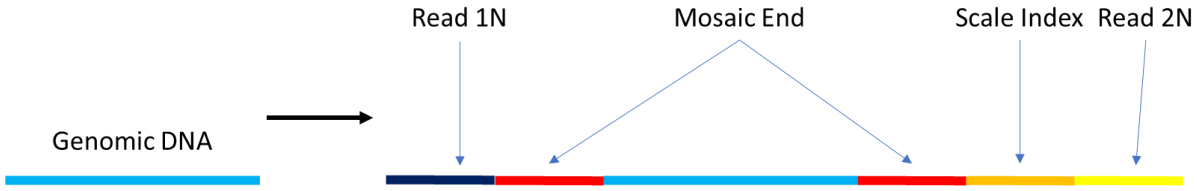


Figure 1. Schematic of the scATAC Pre-Indexing kit with the 10X Chromium Next GEM Single Cell ATAC Reagent Kit and Chromium™ system.

Background

Following nuclei extraction, tagmentation is performed with a set of indexed transposomes (TSMs) containing a DNA transposon adaptor. ScaleBio plates contain 24 uniquely indexed wells; users can load up to 50,000 nuclei in each well.



Following tagmentation nuclei are pooled and up to 100,000 nuclei are loaded into each channel of the Chromium Next GEM Chip. Once encapsulated within GEMs a complementary sequence on the TSM allows for capture and addition of a 10X Barcode specific to the GEM.

GEMs from each channel are then processed in accordance with the Chromium Next GEM Single Cell ATAC protocol. Finally, libraries are PCR amplified with the indexed primers supplied with the ScaleBio scATAC Pre-Indexing kit, allowing for pooling of channels during sequencing.

After sequencing of the libraries, the ScaleBio bioinformatics pipeline can be used to process sequencing data, assigning reads to a sample and an individual nucleus and providing basic ATAC-seq QC metrics. The combination of the Scale Index and 10X Barcode allow multiple nuclei captured within the same GEM to be distinguished the majority of the time. Output files from the pipeline can then be used for further downstream analysis.

Kit Contents

Item	Number/Volume	Storage	Part Number	Lot Number
Indexed TSM Plate (ITP) – Single use; 24 indexed TSMs distributed in wells of 96-well plate	1 5 µl/well	-20°C	110011	
ETB3 – 3x Tagmentation buffer	1 0.6 ml	-20°C	110021	
LB – Loading buffer	1 0.3 ml	-20°C	110041	
Set of iS700 primers	8 25 µl/tube	-20°C	110101	

Additional Requirements

The following materials are required for tagmentation and Chromium indexing. Note that this list does not include items required for nuclei extraction. Similar items from other suppliers may also be appropriate, however optimization may be required.

Item	Supplier	Part Number
Consumables		
Chromium Next GEM Single Cell ATAC Library & Gel Bead Kit v1.1	10X Genomics	16 rxns, 1000175 4 rxns, 1000176
Chromium Next GEM Chip H Single Cell Kit	10X Genomics	48 rxns, 1000161 16 rxns, 1000162
Nuclease free water	Ambion	AM9932
Countess Cell Counting Chamber Slides	ThermoFisher	C10228
High Sensitivity DNA kit	Agilent	5067-4626
8- Strip-tubes	Axygen	PCR-0208-CP-C
1.7 ml microcentrifuge tubes	Axygen	MCT-175-C-S
15 ml conical tube	Sarstedt	62.554.205
Flowmi 40 micron cell strainers for 1000 µl pipette tips	Bel-Art	H13680-0040
BSA Fraction V, 7.5%	Gibco	15260-037
Equipment		
Countess II FL Automated Cell Counter	ThermoFisher	AMQAF1000
Centrifuge for plates with temperature control	Eppendorf Sorvall	022627040 75004240
Benchtop centrifuge with temperature control	Fisher Scientific	13-100-676
Bioanalyzer	Agilent	G2939BA
Disposable reagent reservoir	VWR	89094-658
Magnetic Stand for bead collection	10X Genomics	120250
Thermal cycler for 96 well plate	Bio-Rad	1861096
Microseal B plate sealer	Bio-Rad	MSB1001
Plate shaker	IKA	0003319000
Vortex Genie 2	Scientific Industries	SI-0236
Metal or plastic 96 well PCR rack		

Notes

1. 10X Genomics 20X Nuclei Buffer (2000207) is recommended but nuclei may be maintained on ice in a variety of buffers depending on cell type and chosen extraction method. If using an alternative buffer, please contact ScaleBio to confirm downstream compatibility.
2. If extensive clumping is observed while quantifying nuclei with 0.04% Trypan blue, attempt quantification without the use of Trypan blue as this may reduce clumping and improve accuracy. Additionally, 1% BSA may be included in both Nuclei and Loading buffers to reduce clumping.
3. Filtering of nuclei prior to tagmentation (if not performed during nuclei extraction) and prior to loading of nuclei into 10X Chromium is optional if significant clumping is observed during quantification. Pass nuclei through Flowmi 40 µm filter and quantify again. Note that this will result in some nuclei loss.
4. For ease of workflow, if using all 24 wells for a single sample do not add 5 µl ETB3 to each well. Instead combine 150 µl of nuclei at the appropriate dilution with 150 µl ETB3, then distribute 10 µl of this combination to each well.
5. To ensure even distribution of nuclei, gently flick the tube containing nuclei prior to pipetting and pipette from the center of the volume.
6. Different cell types may require different centrifugation conditions due to size or other variables. It is recommended to optimize centrifugation speed and time for your cell type prior to starting the protocol. Keep in mind that tagmentation also affects nuclei sedimentation properties and we recommend centrifugation at 300 x g for 7 minutes as a safe starting point. Use of a fixed angle centrifuge following tagmentation is recommended to increase visibility of pellet.
7. Be careful to not disturb pellet when aspirating supernatant. Always aspirate with a pipette, not vacuum aspirators. First aspirate majority of supernatant leaving behind a small quantity, then switch to a lower volume pipette to aspirate the remaining volume. If pellet is aspirated, dispense supernatant back into original tube and repeat centrifugation, or cell recovery will be decreased significantly.

Nuclei Extraction

Nuclei extraction procedures will differ based upon cell source and type. Please refer to the following materials for best practices:

Isolation of nuclei from frozen tissue for ATAC-seq and other epigenomic assays (protocols.io)

Single Cell ATAC - 10x Genomics

CG000212_SingleCellATAC_Nuclei_Isolation_MouseBrain_DemonstratedProtocol_RevB.pdf (ctfassets.net)

CG000169_DemonstratedProtocol_NucleiIsolation_ATAC_Sequencing_RevD.pdf (ctfassets.net)

CG000053_CellPrepGuide_RevC.pdf (ctfassets.net)

CG000209_Chromium_NextGEM_SingleCell_ATAC_ReagentKits_v1.1_UserGuide_RevD.pdf (ctfassets.net)

Tagmentation and Indexing

A. Tagmentation

Item	Part Number	Preparation	Storage
ITP	110011	Thaw on ice, vortex ⏱15 sec, centrifuge 2000 x g ⏱5 sec, keep on ice	-20°C
ETB3	110021	Thaw on ice, vortex ⏱15 sec, centrifuge 2000 x g ⏱5 sec, keep on ice	-20°C
Nuclei	N/A	Keep on ice	N/A

☐ 1. Prepare aliquot for counting. Dilute nuclei 10-fold by adding 2 µl of nuclei to 18 µl of 1x Diluted Nuclei Buffer (If utilizing alternative Nuclei Buffer see Note 1). Mix with 20 µl 0.4% trypan blue solution prior to quantifying nuclei using cell counter or hemocytometer. **Perform 2 counts to achieve an accurate estimate.** (See Notes 2 & 3)

☐ 2. Dilute nuclei to desired concentration in 1x Diluted Nuclei Buffer (see "Nuclei Concentration Table", page 11).

☐ 3. Remove the ITP plate from ice and place it into a 96 well PCR rack for improved grip during seal removal.

☐ 4. Carefully remove the aluminum seal, holding the edge of the ITP plate firmly while pulling back diagonally.

☐ 5. Add 5 µl ETB3 to each well followed by 5 µl nuclei dilution (See Notes 4 & 5). **Change pipette tips between wells to prevent cross-contamination of tagmentation indices.**

☐ 6. Mix by gentle pipetting up and down 3 times.

☐ 7. Seal the ITP plate using Microseal B. **A poor seal can result in evaporation and loss of sample.**

☐ 8. Incubate at 37°C for ⏱60 min in thermocycler block with a heated (47°C) lid.

☐ 9. While incubating plate, thaw Loading Buffer (LB) on ice.

B. Stop Tagmentation

Item	Part Number	Preparation	Storage
LB	110041	Thaw on ice, vortex ⏲15 sec, centrifuge 2000 x g ⏲5 sec, keep on ice	-20°C

☐ 1. Remove the ITP plate from the thermocycler.

☐ 2. Incubate the plate on ice for ⏲5 min before removing the seal from the plate.

☐ 3. Pipette up and down 3 times to resuspend nuclei prior to removing and pooling contents of each well, and transfer all volume to a fresh 1.7 ml microcentrifuge tube.

☐ 4. Centrifuge nuclei at 300 x g for ⏲5 min at 4°C. (See Note 6)

☐ 5. Completely remove the supernatant **without disturbing nuclei pellet**. (See Note 7)

☐ 6. Add 50 µl of the LB buffer and fully resuspend nuclei pellet by inverting and gently flicking. If pellet is not fully resuspended perform gentle pipetting up and down 5-10 times.

☐ 7. Prepare aliquot for counting. Dilute nuclei 10-fold by adding 2 µl of nuclei to 18 µl of LB buffer. Mix with 20 µl 0.4% trypan blue solution prior to quantifying nuclei using cell counter or hemocytometer. **Perform 2 counts to achieve an accurate estimate.** (See Notes 2 & 3)

☐ 8. Prepare 100,000 nuclei for loading in 15 µl of LB buffer by preparing a nuclei dilution of 7,142 nuclei/µl. This 15 µl will be combined with 60 µl of 10X Master Mix prior to loading of 10X Chromium. **If not loading 100,000 per channel, divide desired loading by 14 to calculate appropriate nuclei concentration per µl.**

C. 10X Chromium Indexing

☐ 1. Proceed as described from Step 2, page 24 of Chromium Next GEM Single Cell ATAC Reagent Kits v1.1 (CG000209 RevD) protocol until GEM Incubation (step 2.5.a)

A. ***Limit the number of cycles to 4 at step 2.5a.***

☐ 2. Proceed as described in standard protocol until Sample Index PCR (step 4.1.c)

A. Instead of 10X Single Index N Set A reagent use ***2.5uL of one of ScaleBio S700P reagent per 10X Chromium channel. This will allow for pooling of samples from different channels for sequencing in the same sequencing run.***

B. ***Limit the number of PCR cycles to 8 at step 4.1c.***

Appendix

Nuclei Concentration Table

Number of Nuclei per Well	Required Nuclei Concentration (Nuclei/µl)	Approx. Final Output of Well
20,000	4,000	5,000
25,000	5,000	6,250
30,000	6,000	7,500
35,000	7,000	8,750
40,000	8,000	10,000
45,000	9,000	11,250
50,000	10,000	12,500

Comparative results are obtained with 20,000 – 50,000 nuclei per ITP well, exceeding these limits is not recommended.

iTSM Index Layout

	1	2	3
A	GAACCGCG	AGGTTATA	TCATCCTT
B	TGGCCGGT	CAATTAAC	ATAATGTG
C	TCTGTTGG	CTCACCAA	TATTAGCT
D	ATGTAAGT	GCACGGAC	GGTACCTT
E	ATCCACTG	GCTTGTCA	CAAGCTAG
F	TAAGTGGT	CGGACAAC	ATATGGAT
G	GCTCATTG	ATCTGCCA	CTTGGTAT
H	GATCTATC	AGCTCGCT	CGGAACTG

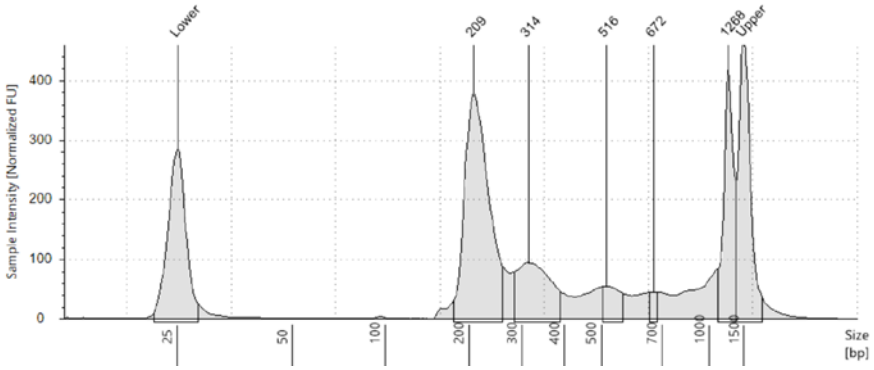
ScaleBio Indexing Amplification Primers

Each S70XP primer contains an equal mix of 4 indexed primers in order to ensure equal base distribution and optimize sequencing quality.

PRIMER	SEQUENCE 1	SEQUENCE 2	SEQUENCE 3	SEQUENCE 4	PART NUMBER
S701P	GGTCACCT	TAGACATC	CTCTGTGA	ACAGTGAG	110111
S702P	CAGAGAAT	AGCGCTTG	GTTCACGA	TCATTGCC	110121
S703P	AGGCCGAA	CCATAAGC	TACAGCTT	GTTGTTCG	110131
S704P	TATACTGA	CCGTGGAT	GGACACTG	ATCGTACC	110141
S705P	GGTCCAGA	CAAGGTCT	ACCTTGTG	TTGAACAC	110151
S706P	GGAACTAG	CTTCGAGC	TCCTAGCT	AAGGTCTA	110161
S707P	TCGGTACA	GGAACGGT	CATTGCTG	ATCCATAC	110171
S708P	GGTTCATG	CTAAGGAT	TACCATCA	ACGGTCGC	110181

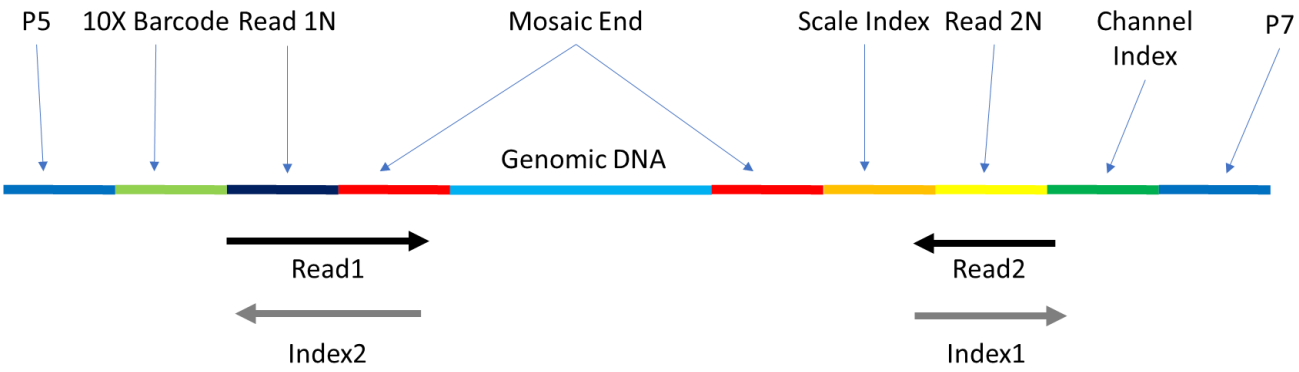
Post Library QC

Representative Scale ATAC library size distribution on TapeStation High Sensitivity D1000 ScreenTape



Sequencing Diagram and Recommendations

Standard sequencing recipe with standard sequencing primers, no PhiX spike in.



Read	Read 1	Index1 (i7)	Index2 (i5)	Read2	Total
Purpose	Genomic DNA	Channel Index	10X Barcode	Scale Index, ME, Genomic DNA	
Length (bp)	50	8	16	(8,19,50) 77	151

Analysis Considerations

- Each single cell corresponds to a unique combination of *Scale index* and *10X Barcode*
    - A single partition can contain multiple unique cells with different *Scale index* sequences
    - If multiple *Channel index* sequences are used for one library, these do not define single cells. The reads should be pooled (as in the 10X Chromium default workflow).
- All barcode sequences should be error-corrected allowing for at least 1 mismatch (*Hamming distance*) between read and barcode sequence
- The fixed 19 bp *ME`* sequence should be trimmed from read 2 before alignment
- Unique fragments are based on the cell barcode (*Scale index* + *10X Barcode* sequence) and mapping position of both reads.
- The *10X Barcode* is read in the opposite direction from the 10X Chromium default; hence the barcode sequence list should be reverse complemented if using your own pipeline.