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Protocol status: Working
We use this protocol and it's working

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Sinai SCENT TMC - Single Cell Assay for Transposase Accessible Chromatin (ATAC-seq)

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ABSTRACT

The Assay for Transposase Accessible Chromatin (ATAC)-Seq method is a genome-wide, next-generation sequencing (NGS)-based assay that characterizes chromatin states in cell and tissue samples. Specifically, ATAC-Seq is utilized to identify genomic regions with open chromatin states, typically associated with actively transcribing sites, facilitating the identification of transcription factors and determination of nucleosome positioning. As a common entry point for epigenomic analysis, ATAC-Seq probes the molecular mechanisms regulating various cellular processes. Initial findings from ATAC-Seq assays can be further validated and expanded through complementary techniques such as reporter assays, chromatin immunoprecipitations, and DNA methylation assays.

SAFETY WARNINGS

- All personnel must have completed the necessary training, including annual refresher training, on the safe handling of potentially infectious material.
- Personal protective equipment (PPE), which includes gowns, gloves, and protective goggles.

PROTOCOL integer ID: 94625

Procedure

52m

1 Prepare Transposition Mix

1.1 Prepare Transposition Mix (see below) On ice. Pipette mix 10x and centrifuge briefly.

Transposition Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
ATAC Buffer B	2000193	7.0	30.8	61.6
ATAC Enzyme	2000123/ 2000138	3.0	13.2	26.4
Total	-	10.0	44.0	88.0

1.2 Add 10 µL Transposition Mix to a tube of a PCR 8-tube strip for each sample. Centrifuge briefly and maintain 0 °C.

1.3 Refer to Nuclei Concentration Guidelines (see below) to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µL.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

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

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

Volume of Diluted Nuclei Buffer* (µl) = 5 µl - volume of Nuclei Stock (µl)
*Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water)

1.4 Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix. Centrifuge briefly.

1.5 Gently pipette mix the Nuclei Stock. Add the calculated volume of Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 6x (pipette set to 10 uL). DO NOT CENTRIFUGE
THE TUBE.

1.6 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

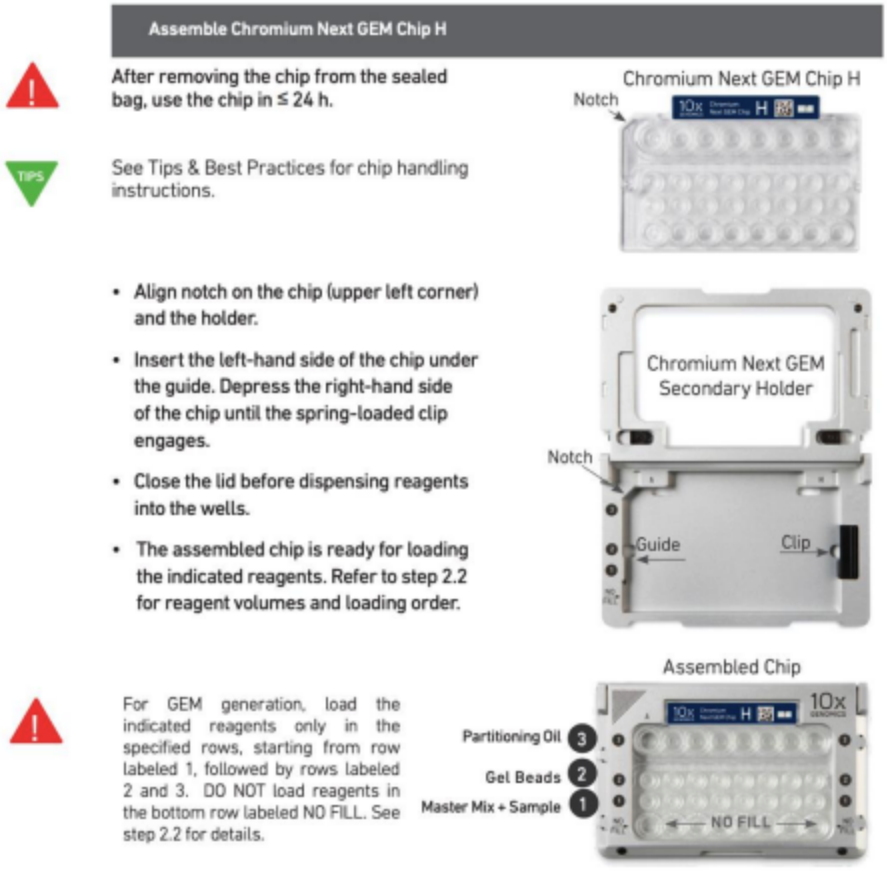
2 GEM Generation and Barcoding

2.1 Prepare master mix On ice. Pipette mix 10x and centrifuge briefly.

Master Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Barcoding Reagent B	2000194	56.5	248.6	497.2
Reducing Agent B	2000087	1.5	6.6	13.2
Barcoding Enzyme	2000125/ 2000139	2.0	8.8	17.6
Total	-	60.0	264.0	528.0

2.2 Place the PCR strip containing Transposed Nuclei on a cooling block.

2.3 Assemble Chromium Next GEM Chip H:



2.4 Load 50% Glycerol into Unused Chip Wells (if <8 samples per chip):
i. 70 ul to unused wells in row labeled 1.
ii. 50 ul to unused wells in row labeled 2.
iii. 40 ul to unused wells in row labeled 3.

2.5 Aliquot 60 µL Master Mix to each tube containing Transposed Nuclei for a total of 75 µL in each tube

2.6 Using P200 multi-channel set to 70 ul, gently mix 5x On ice

2.7 00:00:30 Transfer 70 µL of cell mix to row 1, wait 00:00:30 for cells to prim 1m 30s before adding Gel Beads. Proceed with next step during 00:00:30 priming.

2.8 Vortex Gel Bead Strip for 30 seconds

2.9 Centrifuge the Gel Bead strip for ~5 seconds. Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even. Place the Gel Bead sstrip back in the holder. Secure the holder lid.

2.10 Puncture the foil seal of the Gel Bead tubes. Slowly aspirate 50 ul Gel Beads. Dispense into the wells in row labeled 2 without introducing bubbles.

2.11 Wait 00:00:30 30s

2.12 Add 40 µL of Partitioning Oil to row 3. Remove bubbles

2.13 Attach gasket (notch on top left)

2.14 Place the assembled chip with the gasket into the tray of the Chromium Controller, ensuring th 18m chip stays horizontal. Close the tray and press the play button to begin run (00:18:00). Complete next steps during run.

2.15 Place a PCR 8-tube strip On ice

2.16 When chip run is complete, press the eject button of the Controller to remove the chip. Discard the gasket, open chip holder, and fold the lid back until it clicks to expose the wells at a 45 degree angle.

2.17 Transfer 100 µL of GEMs into PCR strip tube. Pipette slowly. It should take ~20 seconds to pipette GEMs.

2.18 If multiple chips are run back-to-back, cover the GEM-containing strip tube and place On ice for no more than 1 hour.

2.19 Run the following thermocycler program:

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

NOTE: PCR product can be stored at 15°C for up to 18 hours or at −20°C for up to a week, or proceed to the next step immediately.

3 Post GEM Incubation Cleanup

3.1 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.

3.2 Slowly remove and discard 125 µL Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.

3.3 Prepare Dynabeads Cleanup Mix:

Dynabeads Cleanup Mix *Add reagents in the order listed*	PN	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Cleanup Buffer	2000088	182	800.8	1601.6
Dynabeads MyOne SILANE Vortex thoroughly (≥30 sec) **immediately** before adding to the mix. Aspirate the full liquid volume with a pipette tip to verify that the beads have not settled in the bottom of the tube. If clumps are present, pipette mix to resuspend completely. DO NOT centrifuge before use.	2000048	8	35.2	70.4
Reducing Agent B	2000087	5	22	44
Nuclease-free Water	-	5	22	44
Total	-	200	880	1760

3.4 Vortex and add 200 µL of Dynabeads Cleanup Mix to each sample. Pipette mix 5x (pipette set to 200 ul).

3.5 Incubate 00:10:00 Room temperature 10m

3.6 Prepare Elution Solution I. Vortex and centrifuge briefly:

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

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

3.7 At the end of 10 min incubation, place on the 10x Magnetic Separator, high position (Magnet – High) until the solution clears.

3.8 Remove the supernatant.

3.9 Add 300 µL freshly prepared 80% ethanol to the pellet while on the magnet – High. Wait 00:00:30 30s

3.10 Remove the ethanol.

3.11 Add 200 µL 80% ethanol to the pellet. Wait 00:00:30 30s

3.12 Remove the ethanol.

3.13 Centrifuge briefly. Place on the magnet – Low.

3.14 Remove the remaining ethanol.

3.15 Remove from the magnet. Immediately add 40.5 µL Elution Solution I to avoid clumping.

3.16 Pipette mix 15x (pipette set to 40 ul) without introducing bubbles.

3.17 Incubate 00:01:00 Room temperature 1m

3.18 centrifuge briefly. Place on the magnet – Low until the solution clears.

3.19 Transfer 40 µL sample to a new tube strip.

3.20 Vortex the SPRIselect reagent until fully resuspended. Add 48 µL SPRIselect reagent to each sample. Pipette mix thoroughly.

3.21 Incubate 00:05:00 Room temperature 5m

3.22 Centrifuge briefly. Place on the magnet – High until the solution clears.

3.23 Remove the supernatant.

3.24 Add 200 µL 80% ethanol to the pellet. Wait 00:00:30 30s

3.25 Remove the ethanol.

3.26 Repeat steps 3.24 and 3.25 for a total of 2 washes.

3.27 Centrifuge briefly. Place on the magnet – Low.

3.28 Remove any remaining ethanol.

3.29 Remove the tube strip from the magnet. Immediately add 40.5 µL Elution Solution I.

3.30 Pipette mix 15x (pipette set to 30 ul) without introducing bubbles.

3.31 Incubate 00:02:00 Room temperature 2m

3.32 Centrifuge briefly. Place on the magnet – Low until the solution clears.

3.33 Transfer 40 µL sample to a new strip tube.

NOTE: Samples can be stored at 4°C up to 72 hours or at -20°C for up to 1 week, or proceed to the next step.

4 Library Construction

4.1 Prepare Sample Index PCR Mix On ice

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

4.2 Add 57.5 µL of Sample Index PCR Mix to 40 µL sample.

4.3 Add 2.5 µL of an individual Single Index N Set A to each well and record assignment

4.4 Mix by pipetting 15x and centrifuge briefly.

4.5 Run the following thermocycler program:

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~30 min

Step	Temperature	Time
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:00:20 Go to step 2, see table below for # cycles
5	72°C	00:01:00
6	4°C	Hold

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

Cycle Number Optimization Table

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

NOTE: Samples can be stored at 4°C up to 72 hours, or proceed to the next step.

4.6 Vortex to resuspend SPRIselect reagent. Add 40 µL SPRIselect reagent to each sample.
Pipette
to mix 15x.

4.7 Incubate 00:05:00 Room temperature 5m

4.8 Place on the magnet – High until the solution clears.

4.9 Transfer 130 µL supernatant to a new strip tube. DO NOT discard the supernatant.

4.10 Vortex to resuspend SPRIselect reagent. Add 74 µL SPRIselect reagent to each sample. Pipette to mix 15x.

4.11 Incubate 00:05:00 Room temperature 5m

4.12 Place on the magnet – High until the solution clears.

4.13 Discard the supernatant

4.14 Add 200 µL 80% ethanol to the pellet. Wait 00:00:30 30s

4.15 Remove the ethanol.

4.16 Repeat steps 4.14 and 4.15 for a total of 2 washes.

4.17 Centrifuge briefly. Place on the magnet – Low.

4.18 Remove remaining ethanol.

4.19 Remove from the magnet. Immediately add 20.5 µL Buffer EB. Pipette mix 15x

4.20 Incubate 00:02:00 Room temperature 2m

4.21 Centrifuge briefly. Place on the magnet – Low.

4.22 Transfer 20 µL sample to a new tube strip.

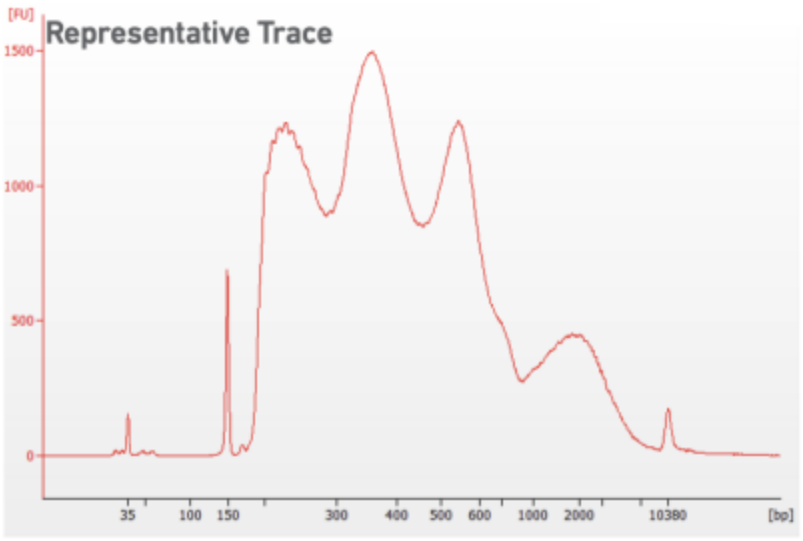
NOTE: Samples can be stored at 4°C up to 72 hours or -20°C for long-term storage.

5 LIBRARY QUANTIFICATION

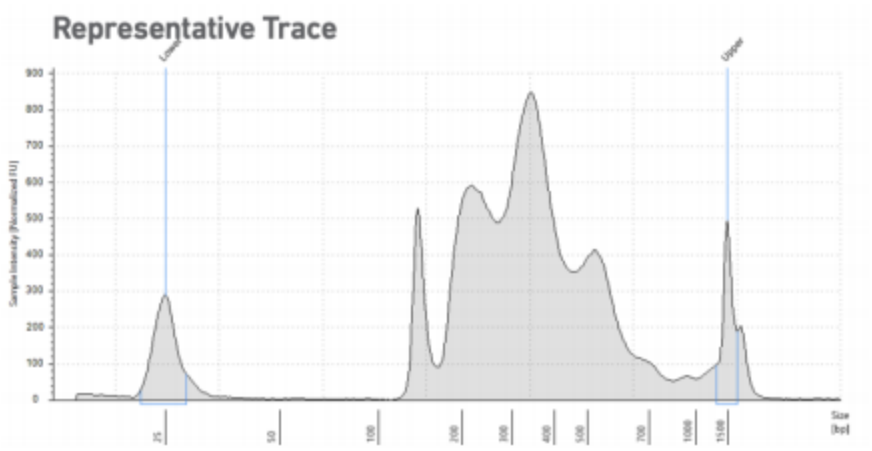
5.1 Qubit - Run 1 µL sample at 1:5 dilution on the Qubit dsDNA HS Assay Kit

5.2 BioAnalyzer/Tapestation

1. EITHER Run 1 µL sample diluted to 3 ng/ul 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.



2. OR Run 2 µL sample diluted to 1 ng/ul on the Agilent Tapestation High Sensitivity D1000 ScreenTape to determine fragment size.



5.3 qPCR

1. Thaw KAPA Library Quantification Kit for Illumina Platforms
2. 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).
3. 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

4. Dispense 16 µL Quantification Master Mix for sample dilutions and DNA Standards into a 96 well PCR plate.
5. Add 4 µL sample dilutions and 4 µL DNA Standards to appropriate wells. Centrifuge briefly.
6. Incubate in a thermal cycler with the following protocol.

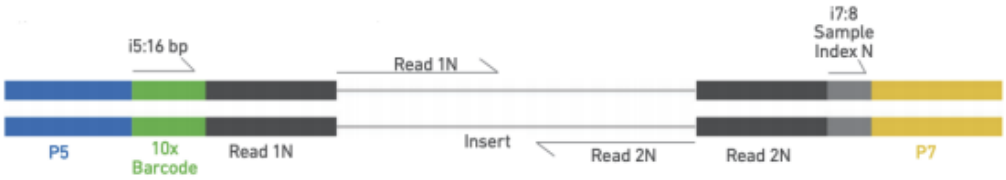
Step	Temperature	Run Time
1	95°C	00:03:00
2	95°C	00:00:05
3	67°C	00:00:30
4	Go to Step 2, 29X (Total 30 cycles)	

7. 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.

6 Sequencing

6.1

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
- 16bp 10x barcode sequence in the i5 read

6.2 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.

- MiSeq
- NextSeq 500/550 (High Output)
- NextSeq 1000/2000
- HiSeq 2500 (Rapid Run)
- HiSeq 3000.4000
- NovaSeq

6.3 Sample Indices

Each i7 sample index in the Single Index Plate Kit N Set A (PN-3000427) is a mix of 4 different sequences to balance across all 4 nucleotides. If multiple samples are pooled in a sequence lane, the sample index name (i.e. Single Index Plate N Set A well ID) is needed in the sample sheet used for generating FASTQs with “cellranger scATAC mkfastq”.

6.4 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

6.5 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.