

Feb 26, 2020 Version 1

sciMAP-ATAC V.1

In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.5r4g58w

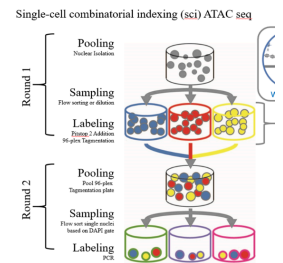
Andrew Adey¹, Casey Thornton¹

¹Oregon Health & Science University



Andrew Adey

Oregon Health & Science University



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.5r4g58w>

External link: doi: <https://doi.org/10.1101/407668>

Protocol Citation: Andrew Adey, Casey Thornton 2020. sciMAP-ATAC. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5r4g58w>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: In development

We are still developing and optimizing this protocol

Created: July 23, 2019

Last Modified: February 26, 2020

Protocol Integer ID: 26140

Keywords: Genomics, Epigenomics, Single Cell, Biotechnology, single cell profiling of chromatin state, throughput single cell genomic assay, single cell genomic assay, resolved single cell profiling, cell combinatorial indexing on microbiopsy, cell atac, accessible chromatin, transposase accessible chromatin, chromatin state, cell localization, cell combinatorial indexing, heterogeneity of cell state, cell data, understanding complex tissue, cell data of equivalent quality, interconnected cell, high density multiregional sampling, cell state, complex tissue, cell

Abstract

High-throughput single cell genomic assays resolve the heterogeneity of cell states in complex tissues, however, the spatial orientation within the network of interconnected cells is lost. As cell localization is a necessary dimension in understanding complex tissues and disease states, we present a tool for highly scalable spatially-resolved single cell profiling of chromatin state. We use high density multiregional sampling to perform single-cell combinatorial indexing on Microbiopsies Assigned to Positions for the Assay for Transposase Accessible Chromatin (sciMAP-ATAC) to produce single-cell data of equivalent quality to non-spatial single-cell ATAC-seq.



Materials

MATERIALS

- ✕ Magnesium Chloride **Fisher Scientific Catalog #AC223210010**
- ✕ IGEPAL-CA630 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I3021 SIGMA-ALDRICH**
- ✕ Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML**
- ✕ Tween-20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P-7949**
- ✕ Sodium Chloride **Fisher Scientific Catalog #S271-3**
- ✕ Agencourt Ampure XP **Beckman Coulter Catalog #A63880**
- ✕ 4,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) **Thermo Fisher Scientific Catalog #D1306**
- ✕ Embedding base molds **Fisher Scientific Catalog #22-363-553**
- ✕ Jung tissue freezing medium (Leica Microsystems) or OCT compound (TissueTek)
- ✕ Cell strainer, 35 μ m **Corning Catalog #352235**
- ✕ Pierce Protease Inhibitor Tablets, EDTA-Free **Thermo Fisher Scientific Catalog #A32955**
- ✕ Tris-HCl **Life Technologies Catalog #AM9855**
- ✕ Superfrost Plus Microscope Slides **Thermo Fisher Scientific Catalog #4951PLUS4**
- ✕ 1X PBS, cell culture grade **Thermo Fisher Scientific**
- ✕ Potassium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9541**
- ✕ EDTA **Invitrogen - Thermo Fisher Catalog #AM9261**
- ✕ Qiagen Protease **Fisher Scientific Catalog #NC9221823**
- ✕ Pitstop 2 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #SML1169-5MG**
- ✕ Nextera DNA Flex Library Prep **Illumina, Inc. Catalog #20018705**
- ✕ QIAquick PCR Purification Kit **Qiagen Catalog #28106**
- ✕ Uniquely Indexed Transposomes
- ✕ Sci- Barcoded PCR Primers
- ✕ Pitstop 2 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #SML1169**

Tween-20: working stock is 10% (100X). Aliquots are stored at 4C.

IGEPA-630: Prepare 10% (v/v) stock made with diH₂O, store at Room Temperature (RT).

DAPI: Resuspend to 5 mg/mL in diH₂O. Aliquot and store at -20C.

Pitstop2: Resuspend in 3mM in DMSO. Aliquot and store at -20C.

Supplies List:



- 96-well PCR plates (Eppendorf, 951020427)
- 35 um cell strainer (VWR, 21008-948)
- High Sensitivity DNA Chip (Agilent, 5067-4627)

Instrument List:

- Table top centrifuge cooled to 4C with rotors for spinning 1) 96-well plates, and 2) 15 mL falcon tubes at 600 rcf
- Fluorescence Activated Cell Sorter (FACS), we use Sony SH800S
- Thermomixer with 96 well plate adapter (55C incubations at 300 rpm), we use Eppendorf Themomixer C
- Real-Time PCR instrument (Bio-Rad CFX Connect)
- DNA fluorometer or spectrophotometer (Qubit Fluorometer 2.0 is used in this protocol)
- Agilent Bioanalyzer
- Sequencing: NextSeq 500 using custom chemistry protocol

Troubleshooting

Before start

Cryopreserved tissue sections: Prepare prior to sp-sciATACseq protocol start. Refer to "Cryopreserved tissue sectioning" protocol

Uniquely indexed transposomes (8 uM): Prepare and load prior to sp-sciATACseq protocol start. Refer to "sci Transposase Loading" protocol.

Sp-sci barcoded PCR primers: Prepare prior to sp-sciATACseq protocol start. Refer to "sci Barcoded PCR Primer Preparation" protocol.



Prepare Nuclei Isolation Buffer

1


Construct 50mL **Nuclei Isolation Buffer** (NIB):

Final Concentration	Stock Concentration	Volume of Stock
10 mM Tris HCl, pH 7.5	1M Tris-HCl, pH7.5	500 uL
10 mM NaCl	5M NaCl	100 uL
3mM MgCl ₂	1M MgCl ₂	150 uL
0.1 % Igepal	10% Igepal	500 uL
0.1 % Tween	10% Tween	500 uL
ddH ₂ O		to 50mL (add 48.25mL)

Note

OPTIONAL: To prevent protease degradation, we also add 2 tablets of **Pierce Pretease Inhibitor Tablets, EDTA-Free** to NIB following construction. We then vortex to fully dissolve tablets.

**Note**

NIB is stable at  4 °C for at least 1 month without noticeable degradation in library quality or nuclei dissociation ability.

Store NIB on ice throughout nuclei dissociation and preparation of tagmentation plates.

Isolate nuclei

2 **Nuclei from cryopreserved histological sections**

If sample is sourced from micro biopsy of a cryopreserved histological section, dissociate cells using NIB incubation and trituration (described below).

Note**Note**

Isolation of nuclei is dependent on the sample being used. And optimization should be performed. Below we list two example nuclei isolation protocols to act as general use for cell culture and primary tissue samples. Tissue should follow a dounce homogenization protocol, while liquid cell cultures can be pelleted and resuspended directly in NIB.

This protocol is optimized for brain tissue micro biopsies. Additional optimization may need to be performed for other tissues.

1. Prepare 96-well plate(s) for micro biopsy punches

- Pipette 100 uL NIB into each well. Number of wells corresponds to number of punches to be collected.
- Seal plate and store on ice until ready to collect micro biopsies

2. Prepare instruments & tissue for collecting micro biopsies.

- Transfer cryopreserved tissue sections from -80C freezer on to dry ice in an insulated container
- Load Palkovitz punch handle with selected diameter punch (options: 250 um, 500 um, 750 um, 1 mm, 1.25 mm)
- Prechill Palkovitz punch by placing the punch in dry ice

3. Collect microbiopsies **in a cryostat at -20C**

- Place tissue cryosection slide in cryostat and allow ~1 min to acclimate
- Locate region of interest and collect punch
- Deposit punch in well of 96-well plate by depressing punch plunger. (Ensure that punch enters well)
- Repeat for each region to be resected. Place each new punch in new well
- Reseal 96-well plate(s)

Note

Note: Keep a record of 1) slide number, 2) punch location, and 3) well ID for each punch. Annotating image at cryostat works well.

4. Dissociate and wash microbiopsies

- Shake plate on ice for 1 hour at 80 rpm
- Using a multi-channel pipettor, triturate each well 30x.

Note

Note: Pipette gently in order to reduce bubbles and to prevent nuclei shearing

- Spin down plate for 10 min at 500 rcf at 4C
- Using a multi-channel pipettor, aspirate 90 uL of supernatant.

Note

Note: Pellet will not be visible. Be careful to not touch sides of bottom while drawing off supernatant.

5. Dilute microbiopsy nuclei to desired concentration

**Note**

Note: We find that for microbiopsy punches from 200 um thick tissue /250 um biopsy punch results in (thousand nuclei):

Min: 6, 1st Q: 12, Median: 15, Mean: 16.85, 3rd Q: 22.25, Max: 29

We want 10 uL nuclei well. Each punch dissociation can be split into 4 wells (4.2K nuclei/rxn).

Therefore, we want 40 uL of 4,200 nuclei/10 uL:

$$C1V1 = C2V2$$

$$(1,685 \text{ nuclei/uL})(10\text{uL}) = (421.25 \text{ nuclei/uL})(x \text{ uL})$$

$$x = 40 \text{ uL}$$

$$\text{Volume to add: } 40 \text{ uL} - 10 \text{ uL (residual volume)} = \mathbf{30 \text{ uL}}$$

Final concentration of Pitstop 2 should be 70 uM in 40 uL of resuspended nuclei. Therefore:

$$C1V1 = C2V2$$

$$(3000 \text{ uM})(x \text{ uL}) = (70 \text{ uM})(30 \text{ uL}); x = 1.43 \text{ uL}$$

Therefore, for each well, add: (1.4 uL 3 mM Pitstop 2 + 28.6 uL NIB) = 30 uL of 70 uM Pitstop 2 NIB

This should be done by making a master-mix. Given 1 plate (96 wells), prepare a master-mix for 120 wells:

$$(168 \text{ uL } 3\text{mM Pitstop 2} + 3,432 \text{ uL NIB}) = 3600 \text{ uL of } 70 \text{ uM Pitstop 2 NIB, for one plate.}$$

- Prepare 70 uM Pitstop 2 + NIB master mix: For one plate, combine 168 uL 3 mM Pitstop2 & 3,432 uL NIB
- Add 30 uL of 70 uM Pitstop 2 NIB master mix to each well and triturate to resuspend cells

6. Split punches into multiple plates

- Split 40 uL of resuspend cells into 4 new 96-well (DNA/protein Lo-bind) plates with 10uL diluted cells/well.

Note

Note: Make sure to keep well ID consistent between plates.

96-plex Tagmentation

3 Prepare tagmentation plate





Add the following reagents to diluted nuclei in 96-well plate(s) (DNA and Protein Lo-bind):


Prepare 70uM Pitstop 2 + 2X TD buffer mastermix for one plate: 28 uL 3mM Pitstop2 + 1,200 uL 2X TD


Add  10 μ L 70uM Pitstop 2/TD buffer (2X) to each well

Add  1 μ L 8uM uniquely indexed transposase to each well

Spin down plate for  00:01:00 min at 500 rcf at  4 °C

4 **Tagmentation**

Seal plate and incubate at  55 °C with gentle shaking (300 rpm on themomixer) for

 00:15:00

Place plate on ice immediately to stop reaction.

- Keep samples on ice to prevent over-transposistion and nuclei lysis.

5 **Pool all wells for second sort**

Pool all wells into 15mL conical tube, while maintaining everything on ice.

Add 2uL/per mL pooled sample of DAPI (5mg/mL) and bring to sorter for second sort.

96-plex PCR

6 **Preparing Second Plate of Transposase Neutralization Buffer (8.5uL/well):**

	Final Concentration	Stock Concentration	Volume of Stock



	0.59 mg/ mL	20 mg/ mL BSA	0.25 uL
	0.05 9% (w/v)	1% SDS (w/v)	0.5 uL
	to 8.5 uL	ddH 2O	7.75 uL

Per well reagent volumes.

Add 2.5 uL of 10 uM i5 Indexed PCR Primer and 2.5 uL of 10 uM i7 Indexed PCR Primer prior to sort.

2nd Sort Protocol

7 Flow sort single nuclei based on DAPI gate

Note

Sort **X** nuclei per well (**X** is dependent on number of wells tagged in first sort, as a linear trend)

- 96 wells (1 plate) = 22 nuclei/well for PCR
- 144 wells (1 and 1/2 plates) = 33 nuclei/well for PCR
- 192 wells (2 plates) = 44 nuclei/well for PCR etc...

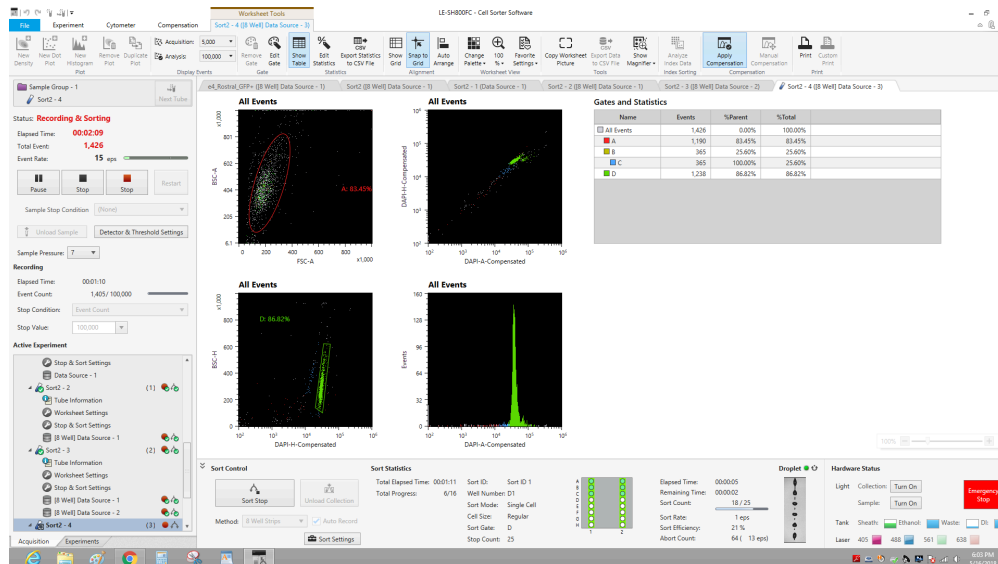
Using the same gates as first sort, sort **X** nuclei per well into prepared second plate with modified sort settings:



- "Single cell" rather than "Normal"

This leads to a higher abort count (less efficient sorting) but is more precise in quantification

- Keep sorted samples on ice to prevent transposases cross-reacting with other nuclei.

Example gating strategy using the Sony SH800 Flow sorter:



Spin down plate at 500 rcf for  00:03:00 min at  4 °C to ensure nuclei are properly suspended in solution.

Note

Volume added, even by sorting 100 nuclei is minimal in our hands and does not require concentration adjustments.

Transposase Denaturation

8 Transposase Denaturation

Denature remaining transposase in sorted nuclei using SDS mixture on Eppendorf Thermocyclers.

 55 °C for  00:20:00 min

9 Amplifying single cell libraries

**Note**

Nextera PCR Mater Mix currently produces the highest quality libraries. An alternative master mix using Kapa Hifi Non-Hotstart has been developed and produces good results.

STEP CASE**Using Nextera PCR Master Mix** 7 steps

10 Add 13.5 uL PCR Master Mix to each well

🧪 7.5 µL NPM

🧪 0.25 µL 100X SYBR Green I

🧪 5.5 µL dH₂O

Perform Real-time PCR on the Bio-Rad CFX Connect:

Temperature (C)	Time	
72	5 min	
98	30 sec	
98	30 s	x30 Cycles
55	30 s	
72	1 min	
	PLATE READ	
72	20 sec	

PCR protocol for Kapa Hifi Non-Hotstart Library Amplification

Pull once majority of well begin to plateau. Sci-ATAC libraries amplify between 14-22 cycles dependent on nuclei per well.

Store libraries at 🧊 4 °C for 6 months or 🧊 -20 °C forever


Library Clean-up and Quantification

11 Pool post-PCR Product

Pool 10 uL from each well into 15mL conical tube.


12 Concentrate DNA via column clean up

Run full pool volume through Qiaquick PCR purification column following manufacturer's protocol.


Elute in  50 µL 10 mM Tris-HCl pH 8.0

13 Clean by size selection with SPRI beads

Perform a 1X SPRI bead size selection (selecting for DNA > 200 bp).

Add  50 µL 18% PEG SPRI Beads to column elution, once beads are at room temperature.


Let mixture incubate at room temperature for  00:05:00 min

Place tube on magnetic rack and wait for magnetic beads to pellet and elution to fully clear (roughly  00:02:00 min)

Remove full volume of elution without disrupting bead pellet.


Resuspend bead pellet in freshly prepared  100 µL 80% ethanol (v/v)


Remove full volume of elution without disrupting bead pellet.

Let beads fully air dry (roughly  00:08:00 min)

- Beads will first lose sheen, and then begin to form cracks.

Resuspend beads off magnetic rack in  31 µL 10 mM Tris-HCl pH 8.0

Let mixture incubate at room temperature for  00:05:00 min for DNA to fully become suspended.

Place tube on magnetic rack and wait for magnetic beads to pellet and elution to fully clear (roughly  00:20:00 min)

Remove full volume of elution without disrupting bead pellet and move to clean tube.

14 Qubit DNA HS Quantification



Quantify DNA concentration with 1uL eluted sample on Qubit DNA High-sensitivity kit following manufacturer's protocol.

15 **Agilent DNA HS Bioanalyzer Quantification**

Dilute sample to 4 ng/uL based on read out of Qubit by addition of 10mM Tris-HCl pH 8.0.

Run 1 uL sample on Agilent DNA HS Bioanalyzer following manufacturer's protocol.

Sequencing

16 **Custom Nextseq500 Chemistry Protocol**

Custom primers and sequencing protocol for sci-atac libraries.