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## sci-ATAC-seq HTAN

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Andrew Adey<sup>1</sup>, Andrew Fields<sup>1</sup>, Casey Th<sup>1</sup>, Ryan Mulqueen<sup>1</sup>, Sarah Vitak<sup>1</sup>, Eleonora Juarez<sup>1</sup>

<sup>1</sup>Oregon Health & Science University



**Andrew Adey**

Oregon Health & Science University

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**We are still developing and optimizing this protocol**

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## Abstract

Single-cell combinatorial indexing ATAC-seq (Sci-ATAC-seq) workflow fro human cells/tissue.

This protocol is the up-to-date Adey Lab protocol that incorporates the use of Pitstop2



## 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**
- ✕ Pierce Protease Inhibitor Tablets, EDTA-Free **Thermo Fisher Scientific Catalog #A32955**
- ✕ Tris-HCl **Life Technologies Catalog #AM9855**
- ✕ 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

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

**IGEPAL-630:** Prepare 10% (v/v) stock made with diH<sub>2</sub>O, store at Room Temperature (RT).

**DAPI:** Resuspend to 5 mg/mL in diH<sub>2</sub>O. 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)
- Thermomixer (55C incubations at 300 rpm)
- Real-Time PCR instrument (Bio-Rad CFX Connect)
- DNA fluorometer or spectrophotometer (Qubit Fluorometer is used in this protocol)
- Agilent Bioanalyzer
- Sequencing: NextSeq 500 using custom chemistry protocol

## Before start

Uniquely indexed transposomes (8 uM) should be prepared and loaded prior to sci-ATACseq protocol start. Refer to "sci Transposase Loading" protocol.

Sci- barcoded PCR primers should be prepared prior to sci-ATACseq protocol start. Refer to "sci Barcoded PCR Primer Preparation" protocol.

## Prepare Buffers and Pre-chill

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Final Concentration	For Construction
10 mM Tris, pH 7.5	500 uL of 1M Tris, pH7.5
10 mM NaCl	100 uL of 5M NaCl
3mM MgCl <sub>2</sub>	150 uL of 1M MgCl <sub>2</sub>
0.1 % Igepal	500 uL of 10% Igepal
0.1 % Tween	500 uL of 10% Tween
ddH <sub>2</sub> O	to 50 mL
Protease Inhibitor	2 tablets

Table 1. Nuclear Isolation Buffer (NIB) formulation.

Note: NIB keeps for ~1 month at 4C. For most preparations, 15 mL is sufficient.

## Nuclear Isolation

2


### Note


#### Note


Isolation of nuclei is dependent on the sample being used. Tissue should follow a dounce homogenization protocol, while liquid cell cultures can be pelleted and resuspended directly in NIB.



Generalized nuclear isolation steps:

1. Pellet  10 mL liquid cultured cells in  4 °C centrifuge at 500 rcf for



 00:10:00 min

2. Aspirate pellet and resuspend in  2 mL NIB

3. Incubate on ice for  00:10:00 min



3. Pellet liquid cultured cells in  4 °C centrifuge at 500 rcf for  00:05:00 min



4. Aspirate pellet and resuspend  500  $\mu$ L NIB
5. Run total volume through 35  $\mu$ m filter
6. Add DAPI to final concentration of 15  $\mu$ g/mL (ie  3  $\mu$ L 5 mg/mL DAPI for every 1 mL of sample).

## Perform 1st sort

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1. Per well use  5  $\mu$ L NIB and  5  $\mu$ L TD buffer (2X) from Illumina
2. Sort 2,000-5,000 nuclei into each well using fluorescence activated cell sorting (FACS)

### Note

Nuclei count may vary by prep, but ensure that numbers *within* a prep are constant, ie all wells receive the same number of cells. Keep in ice or in 96-well plate chiller the whole time.

## Tagmentation following 1st Sort

- 4 Spin down 8-well strips in table top centrifuge immediately after sorting finishes.  
Store covered and on ice until the full plate is complete.  
Note: to cover strips as they finish sorting, I tend to tear up aluminum plate covers into single-strip width.  
Addition of Pitstop2 in 2X TD Buffer  
Make sure to test the volume in a well following sort. Sorting a high count of nuclei (2-5K/well increases to volume by 5-10 $\mu$ L, respectively). If unadjusted, this will lower your TD buffer concentration and decrease Tn5 activity.  
In 2X TD Buffer, add Pitstop2 reagent before addition into wells.  
Example: Pitstop2 addition for a 5K nuclei sort (adding 10 $\mu$ L to each well which contains 10 $\mu$ L [NIB+2X TD Buffer] and 10 $\mu$ L of sorted nuclei; 30 $\mu$ L Final Volume).  
Determining concentration of Pitstop2 for TD buffer addition:
  - a.  $C_1V_1=C_2V_2$
  - b.  $(30\mu\text{L Total Volume per well})(70\mu\text{M Final Pitstop Conc.})=(10\mu\text{L 2X TD Buffer})(X \text{ Pitstop2 Concentration})$

♣  $X=210\mu\text{M}$



- a. Determining volume of Pitstop2 to add to 2X TD Buffer mastermix:
  - ♣  $C1V1 = C2V2$
  - ♣  $(210\mu\text{M working concentration})(100\mu\text{L TD mastermix}) = (3000\mu\text{M Pitstop2 stock})(X \text{ Pitstop2 volume})$
  - i.  $X = 7\mu\text{L Pitstop2 stock for each } 93\mu\text{L } 2\text{X TD buffer}$
- Add 1uL 8uM uniquely indexed transposome to each well
- Seal plate and incubate at 55C for 15 minutes with gentle shaking (on eppendorf thermomixer, ~300 rpm).
- Plate plate on ice immediately to stop reaction. Keep samples on ice to prevent over-transposition and nuclei lysis.
- Pool all wells, while maintaining everything on ice.
- Add 2uL/per mL pooled sample of DAPI (5mg/mL) and bring to sorter for second sort.

## 2nd Sort Plate Setup: Preparing Xie Buffer

### 5 Preparing Second Plate Xie Buffer (8.5uL/well):

Volume

Reagent:

6 mL

0.05M Tris-HCl pH 7.8

40 uL

0.5 EDTA

200 uL

1M KCl

200 uL

10% Triton X-100

300 uL

Qiagen Protease

3.26 mL

H2O

10 mL

Total

- 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

- 6
  - Thaw RT-PCR reagents on ice before second sort

- Sort X nuclei per well (X is dependent on number of wells tagged in first sort, as a linear trend)
- 1 plate = 22 nuclei/well
- 1.5 plates = 33 nuclei/well
- 2 plates = 44 nuclei/well etc...
- Using same gates as first sort:
- Sort X nuclei per well with modified sort settings:
  - o "Single cell" rather than "Normal"
  - o This leads to a higher abort count (less efficient sorting) but is far more precise
- As each 8-well strip (containing the Xie buffer) is completed, replace with a new prepared strip.
- Spin down the completed 8-well strip in table-top centrifuge beside the machine.
- Cover the strips and store on ice until all sorting is completed.
- Note: to cover strips as they finish sorting, I tend to tear up aluminum plate covers into single-strip width.
- Keep sorted samples on ice to prevent transposases cross-reacting with other nuclei.

## Transposase Denaturation and PCR setup

- 7
- Spin down 8-well strip tubes
  - Cover plate and hold on ice until sorting is complete.
  - Digest remaining Tn5 and then denature the Qiagen serine protease on Eppendorf Thermocyclers:


•  55 °C  00:20:00

•  70 °C  00:30:00

- 7.1 Add 13.5 ul total of PCR Master Mix to each well:

 5 µL 5X KAPA GC buffer

 0.75 µL 10 mM dntps

 0.5 µL KAPA non-hotstart HiFi

 0.25 µL 100X SYBR Green I

- 8 Perform PCR on the Biorad CFX Connect:





72 °C

00:05:00

98 °C

00:00:30

63 °C

00:00:30

72 °C

00:01:00

Plate Read

72 °C

00:00:20

Pull once majority of wells begin to plateau, for sciATAC libraries amplify between 14-19 cycles

00:00:00

## Library Clean-up and Quantification

- 9 Pool 10uL for each well.
    - Ran full pool volume through Qiaquick PCR column following manufacturer's protocol.
      - Eluted in 32 uL 10mM Tris HCl pH 8
      - Quantified 2 uL in 2:200 dsDNA HS Qubit assay
    - This is to ensure that the library amount will be visible on a gel.
- Use Qubit reading to dilute to ~4ng/uL and run 1uL on HS Bioanalyzer chip
- o Run 1uL of sample at 4ng/uL library dilution on Bioanalyzer High-Sensitivity DNA chip (following manufacturer's protocol)
  - o Quantify library from the range of 100-1000bp
  - o Dilute this down to 1nM concentration for sequencing.