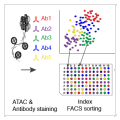

Pi-ATAC

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Human Cell Atlas Method Development Community | HYChangLab

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ABSTRACT

Here we introduce Protein-indexed Assay of Transposase Accessible Chromatin with sequencing (Pi-ATAC) that combines single-cell chromatin and proteomic profiling. In conjunction with DNA transposition, the levels of multiple cell surface or intracellular protein epitopes are recorded by index flow cytometry and positions in arrayed microwells, and then subject to molecular barcoding for subsequent pooled analysis. PiATAC simultaneously identifies the epigenomic and proteomic heterogeneity in individual cells. Pi-ATAC reveals a casual link between transcription factor abundance and DNA motif access, and deconvolute cell types and states in the tumor microenvironment *in vivo*. We identify a dominant role for hypoxia, marked by HIF1A protein, in the tumor microvenvironment for shaping the regulome in a subset of epithelial tumor cells.

EXTERNAL LINK

https://www.biorxiv.org/content/early/2018/04/27/310359

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

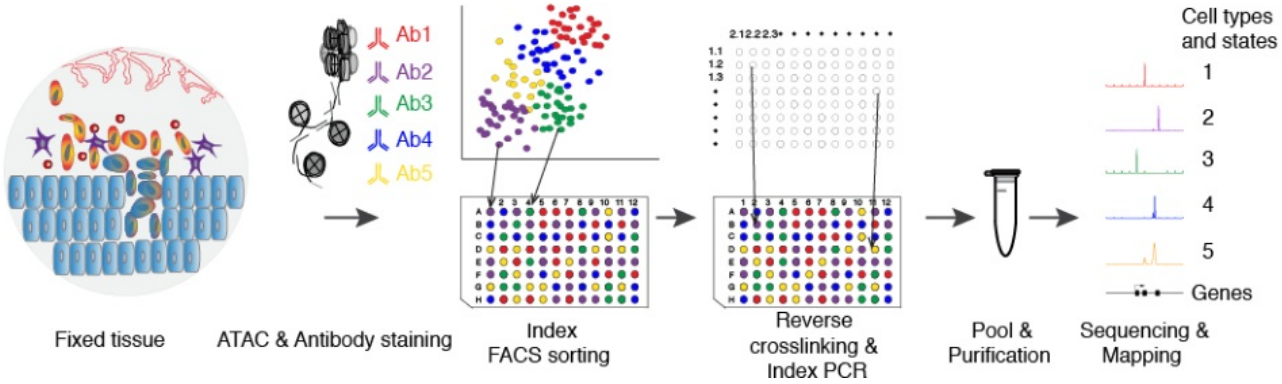
Joint single-cell DNA accessibility and protein epitope profiling reveals environmental regulation of epigenomic heterogeneity
Xingqi Chen, Ulrike Litzenburger, Yuning Wei, Alicia N. Schep, Edward L. LaGory, Hani Choudhry, Amato J. Giaccia, William J. Greenleaf, Howard Chang
doi: https://doi.org/10.1101/310359

PROTOCOL STATUS

Working
We use this protocol in our group and it is working

GUIDELINES

Overview



MATERIALS

NAME	CATALOG #	VENDOR
Nextera DNA library preparation kit 24 samples	FC-121-1030	Contributed by users

STEPS MATERIALS

NAME	CATALOG #	VENDOR
HBSS – 1% FBS (no collagenase) solution		Contributed by users
PBS		Contributed by users
2%PFA		Contributed by users
Glycine, sodium salt	G-450	Gold Biotechnology
Nextera DNA library preparation kit 24 samples	FC-121-1030	Contributed by users
0.5M EDTA	AM92606	Contributed by users
PBS		Contributed by users
Proteinase K	EO0491	Thermo Fisher Scientific
NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns	M0541L	New England Biolabs
MinElute Reaction Cleanup Kit	28204	Qiagen

SAFETY WARNINGS

For safety warnings and hazard information, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

Prepare before:

2% PFA

1.25 M Glycin

single cell suspension solution: 200 U/ml Collagenase in HBSS-FBS (1%))

Nextera Kit: Illumina #15028212 (containing Tn5, 2X TD buffer)

Permeabilization buffer: 10 mM Tris ph7.5

10 mM NaCl
3 mM MgCl2
add fresh 0.1% NP40 (NOTE: NP40 concentration/other detergents can vary from cell type to cell type)

reverse x-link Buffer: 50 mM Tris-HCl pH 8.0
0.5% Tween20
0.5% Igepal CA-630
5 ng/ml proteinase K

0.5 M EDTA

2x NEBNext High Fidelity PCR MasterMix

Nextera adapters for scATAC (Buenrostro et al, *Nature* **volume 523**, pages486–490 (23 July 2015))

1 NOTE
Please see the "before start" section of the Guidelines for buffers and solutions to prepare prior to start.

Preparation

2 If using tissue:
take out tissue and put on 10 cm dish filled with 10 ml PBS on ice. Weigh

Then make a single cell suspension

3 Remove PBS from dish, but keep on ice.

Single cell suspension

4 Cut tissue in small pieces using a scalpel or razor blade on ice.

5 Add single cell dissociation solution to dish (for most tissues: 200 U/ml Collagenase in HBSS-FBS (1%)).

6 Try to pipet with 10 ml pipet, fill into 15ml tube.

7 Incubate 15 ml tube in 37°C incubator rotating (depending on amount and type of tissue).
37 °C Incubation
00:15:00 Incubation

8 Spin down for 5 min at 1000 rpm.
00:05:00 Spin down

9 Aspirate supernatant.

10 Add 2 ml HBSS –FBS (no collagenase) solution.

HBSS – 1% FBS (no collagenase) solution

2 ml HBSS –FBS (no collagenase) solution

11 Use a glass Pasteur pipet to mechanically disrupt remaining clogs, transfer single cells to a 75µm strainer into a new tube (50 ml or 15 ml).

12 Count cells.

Fix

13 Spin down desired amount of cells.

14 Resuspend in 100 µl PBS (for up to 200 000 cells).

PBS

100 µl PBS

15 Fix cells for 10 min at RT using 100 µl 2%PFA.

2%PFA

100 µl 2%PFA

00:10:00 Fixing cells

16 Quench reaction using 1 volume Glycin (here 200 µl).

200 µl 1.25 M Glycerin

Glycine, sodium salt
by Gold Biotechnology
Catalog #: G-450

17 Spin down.

18 Permeabilize cells using buffer containing 0.1% NP40, no incubation, immediately spin 5 min 1200 rpm.
00:05:00 **Spin**

19 If staining is desired: stain with antibodies here.

ATAC

20 Spin down.

21 ATAC: (for 50,000 cells) 25 µl 2xTD, 22,5 µl H_2O, 2.5 µl Tn5
→ 30 min at 37 °C
25 µl 2xTD
22.5 µl H2O
2.5 µl Tn5
37 °C
00:30:00

Nextera DNA library preparation kit 24 samples
Catalog #: FC-121-1030

22 Stop reaction by adding 40 mM EDTA:
500 mM Stock: 1:12.5

0.5M EDTA
Catalog #: AM92606

23 Spin down for 5 minutes at 1200 rpm.
00:05:00 **Spin down**

24 Resuspend in PBS – place on ice.

PBS

25 Add Proteinase K 200µg/µl to reverse x-link buffer = 1:100 from 20 mg/ml Stock.

Proteinase K
by Thermo Fisher Scientific
Catalog #: EO0491

26 Prepare 96-well plates with 25 µl reverse x-link buffer per well.
25 µl reverse x-link buffer (per well)

27 quick-spin the plates to move all buffer to bottom

FACS and reverse x-link

28 Set up single cell 96 or 384 well sort at a BD FACS AriaII or similar and in Diva software check index sort box.

29 If stained:
measure controls (in bulk): e.g. unstained, FMO, uniquely stained samples.
then measure and record fluorescence intensity.

30 Spin down plates immediately after sort.

31 Use heat sealer for sealing plates.

32 60°C reverse x-link O.N. (min 4 h) in incubator or PCR cycler.
60 °C reverse x-link O.N.
04:00:00 reverse x-link O.N.

PCR and clean up

33 Inactivate Proteinase K for 10 min at 80°C.
80 °C ProteinaseK inactivation
00:10:00 ProteinaseK inactivation

34 PCR: 100 wells/plate: per well: 25 µl NEBNext PCR MM, 2.5 µl Ad1, 2.5 µl Ad2 individual Primerstock concentration: 25 µM
25 µl NEB MM
2.5 µl Ad1
2.5 µl Ad2

NEBNext High-Fidelity 2X PCR Master Mix - 250 rxns
by New England Biolabs
Catalog #: M0541L

35 Cycling conditions:

72°C	5min	
98°C	30 s	
98°C	10 s	20 cycles
63°C	30 s	
72°C	1 min	

36 Cleanup using MinElute vacuum:
pool wells into a reservior,
add PB (6ml /96 well plate)
Wash with PE (10 ml/96well plate),
spin down,
elute in 12 µl EB per 96 well plate.

12 µl EB (per plate)

MinElute Reaction Cleanup Kit
by Qiagen
Catalog #: 28204

37 Library quantification using KAPA and BioAnalyzer. If required library cleanup using PAGE gel