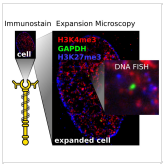


Jun 25, 2021

# Multiplexed, single-cell profiling of histone modifications with SCEPTRE

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Human Cell Atlas Method Development Community

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ABSTRACT

Genome architecture and activity are tightly regulated during an organism's development and function. Histone modifications are thought to contribute to this regulation by acting in combination to specify the activity states of individual genes. However, most methods used to profile these modifications either require a large number of cells or only target an individual histone modification at a time. This protocol uses the method Single Cell Evaluation of Post-TRanslational Epigenetic Encoding (SCEPTRE) to quantify immunolabeled H3K4me3 and H3K27me3 histone modifications at specific non-repetitive genomic loci in single cells using Expansion Microscopy. The protocol can be adapted to target alternative histone modifications or protein assemblies in other cell lines and for other genomic regions of interest.

Reference:
Woodworth,M.A., Ng,K.K.H., Halpern,A.R., Pease,N.A., Nguyen,P.H.B., Kueh,H.Y. and Vaughan,J.C. (2021) Multiplexed single-cell profiling of chromatin states at genomic loci by expansion microscopy. Nucleic Acids Research, 10.1093/nar/gkab423.

ATTACHMENTS

GAPDH_oligo_spreadsheet.xlsx

DOI

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PROTOCOL CITATION

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KEYWORDS

Histone modifications, Expansion Microscopy, Fluorescence in situ Hybridization, Single Cell Profiling

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GUIDELINES

The Single Cell evaluation for Post-TRanslational Epigenetic Encoding (SCEPTRE) protocol is performed as follows:

1. Cells are grown and fixed on coverslips.
2. Histone modifications are immunolabeled.
3. Immunolabeled cells are gelled, digested and expanded according to previous Expansion Microscopy protocols.
4. Genomic loci are Fluorescent *in situ* hybridization (FISH) labeled after expansion.
5. Cells are imaged and the colocalization of immunofluorescent signal at each genomic loci is used as a quantitative measurement of histone mark levels for each locus.

SCEPTRE has been tested and validated for the labeling of histone H3K4me3 and H3K27me3 marks for specific genomic loci in h-TERT RPE1 cells. However, the method can be utilized for labeling other histone modifications or protein assemblies in the nucleus according to notes provided in "Before start". The method is also expected to work with cell lines or primary cells.

For more information about the method, see the below reference:

Woodworth,M.A., Ng,K.K.H., Halpern,A.R., Pease,N.A., Nguyen,P.H.B., Kueh,H.Y. and Vaughan,J.C. (2021) Multiplexed single-cell profiling of chromatin states at genomic loci by expansion microscopy. Nucleic Acids Research, 10.1093/nar/gkab423.

Scripts that can be used for image processing and analysis for SCEPTRE profiling can be found on github at: https://github.com/marcwood13/SCEPTRE_pipeline



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MATERIALS TEXT

Below is a list of materials for profiling of H3K4me3 and H3K27me3 histone marks at *GAPDH* loci in hTERT-RPE1 cells. For targeting of a different set of histone marks, use ChIP-grade antibodies specific to each histone mark of interest. For targeting a different genomic locus of interest, design DNA FISH oligonucleotide probes accordingly.

**primary antibodies:**
Rabbit anti-H3K4me3 (Active motif, 39159)
Mouse anti-H3K27me3 (Active Motif, 61017).

**Secondary antibodies:**
Donkey anti-rabbit (711-005-152) conjugated in-lab to Alexa Fluor 568
Donkey anti-mouse conjugated with Alexa Fluor 488 (715-545-150)

**Enzymes:**
proteinase K (Thermo Fisher Scientific, EO0491)
RNase A (Thermo Fisher Scientific, EN0531)
alcohol oxidase (Sigma-Aldrich, A2404-1KU)
catalase (Sigma-Aldrich, C100)
Phusion Hot-start master mix (New England Biolabs, M0536L)
DNase I (New England Biolabs, M0303A)
Maxima H Minus RT Transcriptase (Thermo Fisher Scientific, EP0752).

**Chemical reagents:**
10× phosphate-buffered saline (PBS, Fisher Bioreagents, BP399-1)
32% paraformaldehyde aqueous solution (PFA, Electron Microscopy Sciences, RT15714)
4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100, Sigma-Aldrich, X100)
Bovine serum albumin (BSA, Rockland Immunochemicals Inc., BSA-50)
Alexa Fluor 568 NHS-ester (Thermo Fisher Scientific, A-20003)
methacrylic acid NHS-ester (MA-NHS, Sigma-Aldrich, 730300)
40% acrylamide aqueous solution (Bio-Rad Laboratories, 1610140)
2% bis-acrylamide aqueous solution (Bio-Rad Laboratories, 1610142)
97% sodium acrylate powder (Sigma-Aldrich, 408220)
ammonium persulfate (APS, Thermo Fisher Scientific, 17874)
tetramethylethylenediamine (TEMED, Thermo Fisher Scientific, 17919)
10× tris-acetate-EDTA (TAE, Fisher Bioreagents, BP2434-4)
guanidine hydrochloride powder (Sigma-Aldrich, G3272)
sodium azide (Sigma-Aldrich, S2002), poly-L-lysine (Sigma-Aldrich, P8920)
sodium bicarbonate (VWR, 470302), formamide (Fisher Chemical, F84-1)
20× saline sodium citrate (SSC, Sigma-Aldrich, S6639)
50% OmniPur Dextran Sulfate (EMD Millipore, 3730)
Tween 20 (Sigma-Aldrich, P9416), Hoechst 33258 (Sigma-Aldrich, B2883-25MG)
Tris Base (Fisher scientific, BP152-500)
methyl viologen dichloride hydrate (Sigma-Aldrich 856177)
L-ascorbic acid (Fisher scientific, A61-25)

**Additional reagents:**
Dulbecco's modified eagle medium (Gibco, 11995065)
penicillin and streptomycin (Gibco, 15140122)
nonessential amino acids (Gibco, 11140050)
fetal bovine serum (Gibco, 26140079)
0.25% trypsin-EDTA (Gibco, 25200056)

*GAPDH* probe set, adapter and reporters (see supplemental spreadsheet).

SAFETY WARNINGS

32% Paraformaldehyde is toxic and must be handled with care.

Formamide is flammable, toxic and a suspected teratogen. All steps involving warm or heated solutions with Formamide should be handled within a fume hood.



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Guanidine hydrochloride is a  known irritant. Dissolve in water within a fumehood.

Acrlylamide and bis-acrylamide are irritants, carcinogenic and suspected teratogens. Handle with care.

Please refer to the Safety Data Sheet of each reagent for more information on any present safety and/or environmental hazards.

BEFORE STARTING

**Prepare the following solutions:**

**RPE1 Cell culture media (store at 4 °C for ~6 months)**
- Dulbecco's modified eagle medium
- 100 units/mL of penicillin and streptomycin
- 1% (w/v) nonessential amino acids
- 10% (v/v) fetal bovine serum

**Note:** If using a different cell line, use the culture media appropriate for the cell line.

**Permeabilization buffer (store at 4 °C for ~6 months)**
- 1X PBS
- 0.1% (v/v) Triton-X 100

**Blocking Buffer  (store at 4 °C for ~6 months)**
- 1X PBS
- 10% (w/v) BSA
- 3mM Sodium Azide

**Primary solution:**
- 2 µg/mL Rabbit X H3K4me3
- 2 µg/mL Mouse X H3K27me3
- 1X PBS
- 10% (w/v) BSA
- 3mM Sodium Azide

**Note:** Alternative Rabbit and Mouse antibodies can be use to target a different set of histone marks or nuclear protein structures.

**Secondary solution:**
- 3 µg/mL Donkey X Rabbit Alexa568
- 3 µg/mL Donkey X Mouse Alexa488
- 1X PBS
- 10% (w/v) BSA
- 3mM Sodium Azide

**Note:** If using an alternative set of primary antibodies, make sure the secondary antibodies label each primary antibody independently and can be visualized in separate fluorescence channels.

**ExM monomer solution  (store at 4 °C for 1 month)**
- 1× PBS
- 2 M NaCl
- 2.5% (w/w) acrylamide
- 0.15% (w/w)
- N,N'-methylenebisacrylamide
- 8.625% (w/w) sodium acrylate

**Digestion solution (store at 4 °C for ~6 months)**
- 1× TAE
- 0.5% (v/v) Triton X-100
- 0.8 M guanidine HCl
- 8 units/mL proteinase K



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**Hybridization buffer (make fresh for each experiment)**

- 2× SSC
- 50% (v/v) formamide
- 0.1% (v/v) Tween 20

**Hybridization mixture (make fresh for each experiment)**

- 2× SSC
- 50% formamide (v/v)
- 10% dextran sulfate (w/v)
- 0.1% (v/v) Tween 20
- 3 mM sodium azide
- 100nM GAPDH probe set or an oligonucleotide probe library capable of targeting a different locus of interest
- 110nM fluorescent oligonucleotide reporter and adapter

**Note:** If targeting an alternative locus of interest, use ~10-20 nM oligo probe library per kb of targeted genomic region, and 1–1.5× concentration of oligo reporters and adapters to oligo probe library.

**Wash buffer (store at 4 °C for ~6 months)**

- 2× SSC
- 0.1% (v/v) Tween 20

## Cell Culture and Fixation 3d

1 Grow h-TERT RPE1 with **cell culture media** to ~80% confluency.
Step 1 includes a Step case.
**Alt. cell line**

step case

### Alt. cell line

Instead of h-TERT RPE1 cells, an alternative adhesive cell line can be used for this protocol as long as:

1. DNA FISH probes are designed for targeting genomic regions in this cell line.
2. Nuclear expansion matches the expansion factor of the ExM gel recipe used. Proper expansion can be confirmed by comparing nuclear diameters of expanded vs. non-expanded cells.

Suspension cell lines may also work as long as the cell line can be attached to a coverslip before immunofluorescence steps.

2 Trypsinize cells with 0.25% trypsin-EDTA and seed at ~75,000 cells per well on top of round coverslips (no. 1.5, ~12 mm diameter) placed within 24-well culture plates.

3

Grow cells at **37 °C** **in 4% carbon dioxide humidified enviorment** **Overnight** .

4

Briefy rinse cells with **250 µl** of 1× PBS.

6m

5 Fix cells with **250 µl** of cold EtOH:MeOH (1:1) for **00:06:00** at **-20 °C** .

6



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Wash three times with **250 µl** of 1× PBS.

7

Store fixed cells in **250 µl** of 1× PBS azide (1× PBS with 3 mM sodium azide) at **4 °C** .

Immunofluorescence 3d

8 Place fixed RPE1 cells in **250 µl** **permeabilization buffer** for **00:10:00** . 10m

9

Wash cells three times with **250 µl** of 1× PBS.

10 1h

Incubate in **250 µl** of **blocking solution** for **01:00:00** at **Room temperature** .

11

Incubate in **250 µl** of **primary solution** **Overnight ~18 hours** at **4 °C** .

12 10m

Wash the sample with **250 µl** of **blocking solution** three times, **00:10:00** each time.

13 2h

Incubate in **250 µl** of **secondary solution** for **02:00:00** at **Room temperature** .

14 10m

Wash once for **00:10:00** with **250 µl** of **blocking solution**.

15



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Wash three times with **250 µl** of 1× PBS.

16 Post-fix in **250 µl** of freshly made 4% PFA in 1× PBS for **00:10:00** . 10m

17

Wash three times with **250 µl** of 1× PBS.

18

Store immunofluorescently labeled cells in **250 µl** of 1× PBS with 3mM sodium azide at **4 °C** .

Gelation, Digestion and Expansion. 10m

19 Treat immunolabeled cells with **250 µl** of freshly prepared **5 Milimolar (mM)** MA-NHS in 1× PBS for **00:10:00** . 10m

20

Wash samples three times with **250 µl** of 1× PBS.

21 10m

Incubate in **250 µl** of **ExM monomer solution** for **00:10:00** .

22

Remove coverslip with cell sample from 24-well plate and place, cells facing down, on to **50 µl** of **ExM monomer solution** supplemented with 0.15% (w/v) APS and 0.2% (w/w) TEMED.

23 30m

Gel sample at **Room temperature** for **00:30:00** in a sealed container backfilled with nitrogen gas.

24 Gently remove the cell-embedded hydrogel from the 12 mm coverslip.

25 30m



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Incubate cell-embedded hydrogel in **1 mL** of **digestion solution** **Overnight** at **37 °C** .

26

Wash and expand by placing the digested sample in **50 mL** of deionized water, replacing the water every 15-20 minutes for at least three times or until gel sample is ~4× the original size.

27

Store hydrogel sample at **4 °C** in 2× SSC buffer.

DNA Fluorescence in situ hybridization 57m 30s

28 Slice a small (~3.5 mm × 3 mm × 2 mm, equivalent to **20 µl** ) piece of the hydrogel sample.

29 10m

Incubate gel slice in **1 mL** of **hybridization buffer** for **00:10:00** at **Room temperature** .

30 30m

Remove excess buffer and incubate gel slice in **500 µl** of pre-heated **hybridization buffer** for **00:30:00** at **60 °C** .

30.1 Make **50 µl** of **hybridization mixture** and pre-heat to **90 °C** for **00:10:00** . 10m

31 Remove the excess buffer from the gel slice and add **50 µl** of pre-heated hybridization mixture to the gel slice.

32 Denature sample at **90 °C** for **00:02:30** . 2m 30s

33 5m

Hybridize probes to sample **Overnight** at **42 °C** .

34 15m



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Remove excess buffer and wash gel slice by incubating in **500 µl** of preheated **wash buffer** for **00:15:00** at **60 °C** .

35 15m

Remove excess buffer and wash gel slice by incubating in **500 µl** of preheated **wash buffer** for **00:15:00** at **37 °C** .

36 15m

Remove excess buffer and wash gel slice by incubating in **500 µl** of **wash buffer** for **00:15:00** at **Room temperature** .

37 10m

Remove excess buffer and wash gel slice by incubating in **500 µl** of one tenth diluted **wash buffer** for **00:10:00** **On ice** .

38

Store samples at **4 °C** .

## Expansion, imaging and analysis 10m

39 10m

Remove excess buffer, then expand gel slice to ~4× by adding and replacing **500 µl** of deionized water, twice every **00:10:00** at **4 °C** .

40 Place the expanded gel slice on a poli-lysine treated coverslip, cell-side facing down.

41

Image sample with a confocal microscope to obtain 3D image stacks of the nuclei of each cell.

42

Analyze fluorescence intensity of each histone modification at the individual loci to determine the activity state of each locus.



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