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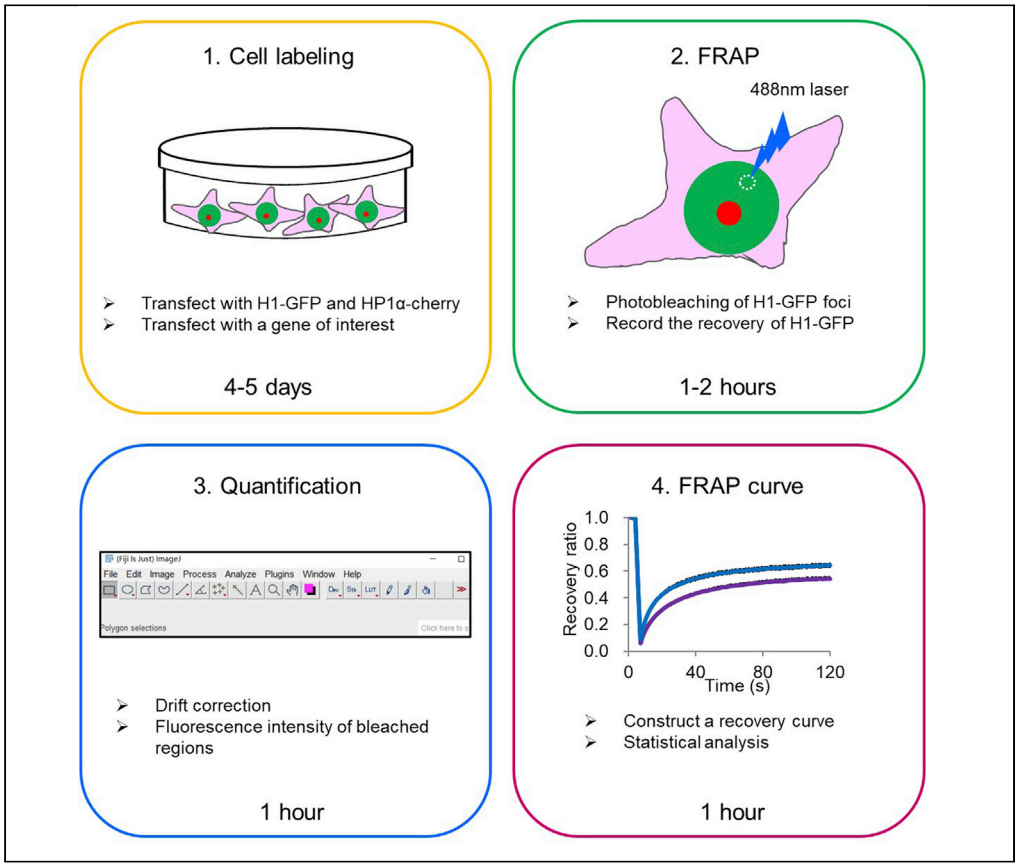
Protocol

Protocol for detecting chromatin dynamics and screening chromatin relaxer by FRAP assay



We describe a fluorescence recovery after photobleaching (FRAP) protocol for assessing the dynamics of heterochromatin/euchromatin and identifying chromatin relaxers for cell fate transition. Here, we developed a system to track heterochromatin foci with HP1α-cherry and performed FRAP assay of H1-GFP to analyze the dynamics of heterochromatin and euchromatin during somatic cell reprogramming. This protocol is used to screen factors that impact chromatin structure, which could also be used to identify chromatin relaxers and repressors in various cell fate transitions.

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Highlights

The FRAP assay detects heterochromatin and euchromatin dynamic in cells, separately

The FRAP assay could be used to screen or identify chromatin relaxers and repressors

Drift in FRAP assay could be corrected with the open-source ImageJ Suite

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Protocol for detecting chromatin dynamics and screening chromatin relaxer by FRAP assay

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SUMMARY

We describe a fluorescence recovery after photobleaching (FRAP) protocol for assessing the dynamics of heterochromatin/euchromatin and identifying chromatin relaxers for cell fate transition. Here, we developed a system to track heterochromatin foci with HP1α-cherry and performed FRAP assay of H1-GFP to analyze the dynamics of heterochromatin and euchromatin during somatic cell reprogramming. This protocol is used to screen factors that impact chromatin structure, which could also be used to identify chromatin relaxers and repressors in various cell fate transitions.

For complete details on the use and execution of this protocol, please refer to Chen et al. (2016) and Chen et al. (2020).

BEFORE YOU BEGIN

The protocol below describes the specific steps for using MEF cells. However, we have also used this protocol in NIH3T3, human fibrosis and other cells.

Plasmid construction

Timing: 1 week

1. The DNA coding full length of mouse H1.4 fusion with EGFP were inserted into pMXs-flag plasmid (pMXs-H1-GFP). The same for construction of pMXs-HP1α -Cherry, containing the full length of mouse HP1α fusion with mCherry (Chen et al., 2016). Other plasmids coding these two genes could also be used. The plasmids coding the genes of interesting could also be constructed in the similar way.

Prepare cells

Timing: 2 days

2. MEF, NIH3T3 or other cell lines could be used. Plate-E cell are used to produce retrovirus. Recovery the cells, such as MEF from liquid nitrogen before the experiment. Culture the cells in normal condition for at least 2 days priority to start the experiment.

⚠ CRITICAL: When primary cells, such as MEF cell were used, too much passage should be avoided, as the senescence of primary cells will affect the test. We usually use 2–4 passage of MEF cells.

KEY RESOURCES TABLE

REGENT OR SOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
PEI	PolyScience	Cat#23966
Polybrene	Sigma	Cat#H9265
Gelatin	Sigma	Cat#ES-006-B
Opti-MEM	Gibco	Cat#31985-023
DMEM	HyClone	Cat#SH30022-2B
FBS	Gibco	Cat#NTC-HK008
NEAA	Gibco	Cat#11140-050
GlutaMAX	Gibco	Cat#35050-061
Penicillin/Streptomycin	HyClone	Cat#SV30010
Confocal dish	WPI	Cat#FD35-100
Precoated confocal dish	WPI	Cat#FD35PDL-100
0.45 μm Sterile filter	Millipore	Cat#SLHVR33RB
Recombinant DNA		
pMXs-H1-GFP	This study	N/A
pMXs-HP1α -Cherry	This study	N/A
Experimental models: Cell lines		
MEF cells	This study	N/A
Human fibroblast	This study	N/A
Platinum-E (plat-E)	A gift from the Fourth Military Medical University	N/A
Software and algorithms		
Zeiss Zen2	Zeiss	Zeiss, commercially available
Fiji ImageJ	Schneider et al., 2012	https://imagej.net/Fiji/Downloads
Multi measure plugin	N/A	https://www.optinav.info/Multi-Measure.htm
StackReg plugin	Thevenaz et al., 1998	http://bigwww.epfl.ch/thevenaz/stackreg/
TurboReg plugin	Thevenaz et al., 1998	http://bigwww.epfl.ch/thevenaz/turboreg/
GraphPad Prism 5.0	GraphPad Software Inc.	https://www.graphpad.com/scientific-software/prism/
Microsoft Excel	Microsoft	Microsoft
Other		
LSM880 confocal microscope with incubator system.	Zeiss	Zeiss, commercially available

MATERIALS AND EQUIPMENT

A laser confocal microscope with incubator system, including temperature, humidity and CO_2 concentration control. We used a Zeiss LSM880 laser confocal with an incubator system for the assay.

Alternatives: Most laser confocal microscopes equipped with humidity control system for live cell imaging could be used, such as Zeiss LSM710, LSM800, LSM880; Leica SP8, SP5; Nikon A1 or Olympus FV1200 and so on.

MEF cell culture medium		
Regent	Final concentration	Volume (ml)
DMEM	87%	435
FBS	10%	50
NEAA	1%	5
GlutaMAX	1%	5
Penicillin/Streptomycin	1%	5
Stored in 4°C within 1 month.		

GraphPad Prism 5.0, 8.0 or other versions could be used in FRAP curve construction. Microsoft Excel could also do all the calculation and FRAP curve construction.

All kinds of transfection reagents could be applied according to the manual, not restricted to PEI.

All kinds of mammalian cells could be used; we use MEFs as a model cell line.

STEP-BY-STEP METHOD DETAILS

Cell labeling

Timing: 4–5 days

The dynamics of chromatin could be detected as the dynamics of linker histone H1. The cells should be labeled with H1-GFP, which will be tested by FRAP. Another marker for heterochromatin HP1α--cherry is used to indicate heterochromatin foci. Then, test genes could be overexpressed or knock-down in the test cell to detect the effect on chromatin dynamics in specific process, such as somatic cell reprogramming and so on (Chen et al., 2016., Chen et al., 2020). It could also be used to test the effect of drugs on chromatin dynamics and so on.

1. Collect virus coding H1-GFP and HP1α-cherry.
   a. Seed Plate-E cell in a 10 cm dish at proper density about 50% confluence the day before transfection.
   b. Transfect the cell with 10 μg plasmid encoding H1-GFP or HP1α-cherry, mixing with 40 μL PEI (1 mg/mL) per dish.
   c. Collect the culture medium containing virus 48 h after transfection and remove cell fragments with a 0.45 μm sterile filter.
   d. Repeat the virus collection once 24 h later after replacing fresh medium. It can be stored in 4°C for about 1 week. For long time storage, it could be kept in −80°C within 1 year.
2. Labeling cells.
   a. Seed the model cells (MEF cells in this protocol) on a 6 cm cell culture dish.
   b. Add polybrene (6 ng/μL in final concentration) to the virus medium to improve the infection efficiency.
   c. Label the MEF cell by adding the 5 mL virus medium containing retrovirus coding H1-GFP and HP1α-Cherry. The amount of H1-GFP should be more than HP1α-cherry, such as 4 mL H1-GFP and 1 mL HP1α-cherry virus medium.
   d. Repeat the infection step with 4 mL fresh virus medium containing only H1-GFP 24 h after the first infection to improve the expression level of H1-GFP (troubleshooting 1).
   e. Replace with fresh culture medium 12 h after the secondary infection.
   f. Check the transfection efficiency with fluorescence microscope 48–60 h after first infection.
3. Test genes overexpression or knockdown.
   a. Seed the MEF cells in a 6-well dish after labeled with H1-GFP and HP1α-cherry.
   b. Infects the model cells with virus encoding test gene 12 h after seeding the model cells.
   c. Repeat the infection step once more to improve the efficiency 24 h after the first infection.

⚠ CRITICAL: The virus amount of H1-GFP and HP1α-cherry could be adjusted to ensure the brightness of H1-GFP, but keep a minimal signal of HP1α-cherry. The labeling efficiency should be checked under a fluorescence microscope to make sure most of the cells are labeled with both H1-GFP and HP1α-cherry.

Pause point: We carried out the FRAP imaging as soon as MEF cells labeled, which should only be used within about 5 passages after labeling with H1-GFP and HP1α-cherry as it would become senescence after several passages. The human fibroblast or other cell lines could be stored in liquid nitrogen and used for a long time after labeling with H1-GFP and HP1α-cherry in step 2.

FRAP imaging

Timing: 2–3 h

The dynamics of chromatin is detected by the recovery rate linker histone H1 after photobleaching (FRAP). The chromatin could be distinguished by heterochromatin marker with HP1α and euchromatin without HP1α-cherry fluorescence. The dynamics of these two groups of chromatins could be recorded separately.

4. Seed the cells on a new confocal dish with glass bottom precoated with 0.1% gelatin or seed on a confocal dish precoated with Poly-D-Lysine (FD35PDL-100, WPI) for cells attaching not well on glass after labeling with H1-GFP and HP1α-Cherry (Figures 1A–1C). Culture the cell in the normal condition at 37°C with 5% CO_2 for 24–48 h before imaging.
5. Put the dish on the stage of the confocal microscope and prewarmed for 5–10 min (Figure 1D). High magnification lens, such as 63× or 100× oil objective (N.A. ≥ 1.4) lens should be used.

6. Find the focus and adjust the setting of the confocal microscope (Figure 1E). To improve the acquiring rate, the frame size could be set as no more than 512×512 pixels. Make sure the acquisition time should be less than 1 s for each frame with a single-channel acquisition model.
   Here is the detail of setup for imaging using Zeiss LSM880 as example (Methods video S1).
   a. Open the software of Zen 3 black edition
   b. Click acquisition bottom.
   c. Choose "Time series", "Bleaching" and "Regions".
   d. Set the argon laser (or 488 nm laser) and 561 nm laser on.
   e. Setup two trackers for imaging, one for EGFP and another for mCherry with proper filter, such as MBS 488/561.
   f. Setup the laser power and gain value. The 488 nm laser power for EGFP should be as lower as possible, such as 0.2% for laser power, while 680 for gain value (using a GaAsP PMT).
   g. Adjust the imaging scale and set the zoom factor as 3 for 63× objective lens or 2 for 100× objective lens. h. Adjust the focus.

7. Acquiring a two-channel image including H1-GFP and HP1α-cherry at first (Figure 2). This image could be used to select interesting regions for bleaching. Select about 2 to 3 regions with 20 pixels in diameter (less than 2 μm) for bleaching. Select 1 region of heterochromatin foci marked with HP1α or 1-2 non-foci regions (Figure 2). The diameter of all regions should be the same.

8. Select a small region for FRAP, such as a round region with 20 pixels (less than 2 μm) in diameter for bleaching. A 488 nm Argon laser could be used to bleach the H1-GFP signal. Adjust the laser power and bleach time length to improve bleaching efficiency. The bleaching efficiency should be at least 70%.
   Here is the detail for FRAP setup (Methods video S1).

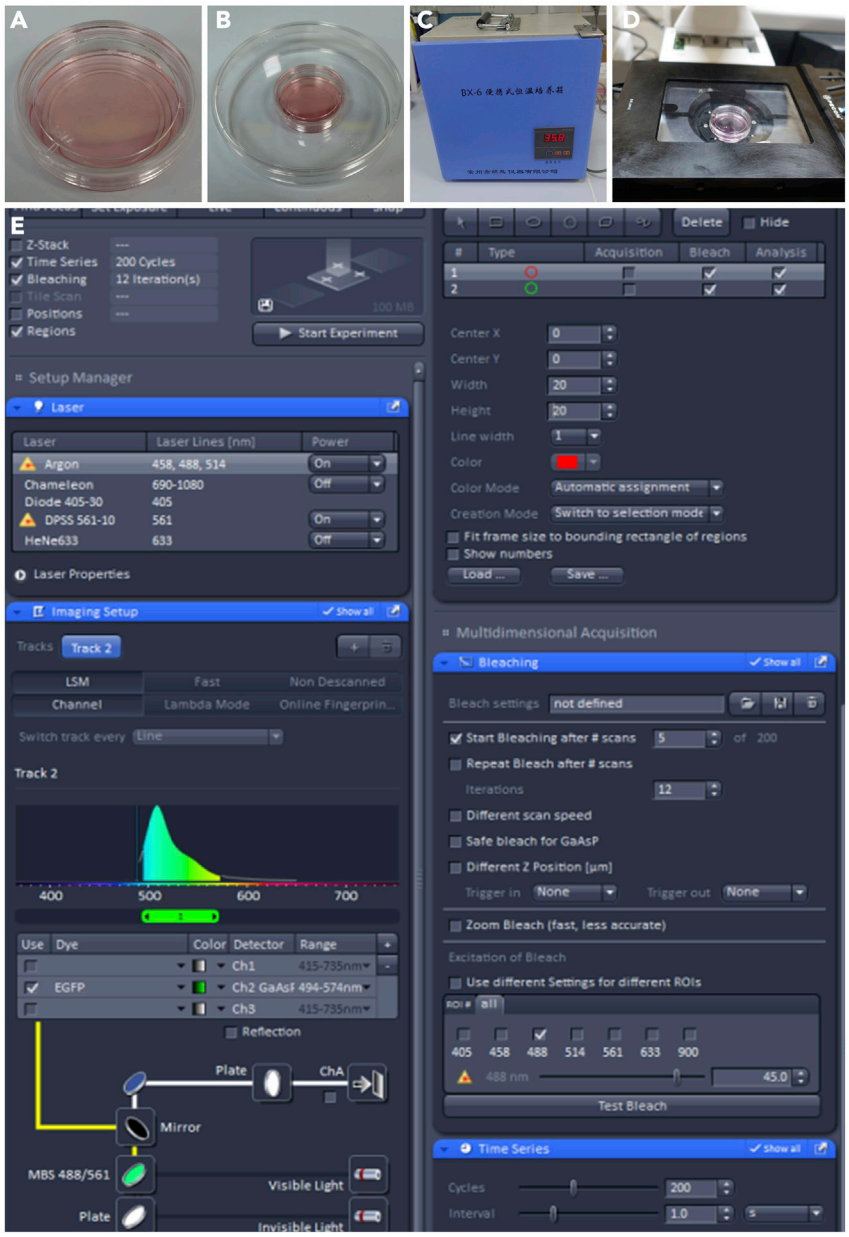

Figure 1. Setup for FRAP imaging

(A) Seed cells on a focal dish with glass bottom.

(B) Put the dish on a 10 cm dish to avoid split out.

(C) Put the dish in a carried-on incubator before imaging.

(D) Put the dish on the stage of microscope with an incubator system.

(E) Setup the confocal microscope for imaging and FRAP.

a. Set the regions for bleaching; Click the round tool to add a round region on the image acquired on step 7. Set both the width and height as 20. The size of regions could be adjusted for different cells or different propose, but it should be kept the same in the same experiments or repeats in different groups.

b. Unfold the bleaching tool bar, and set the "Start Bleaching after # scans" as 5 and "Repeat bleach after # scans" as 12.

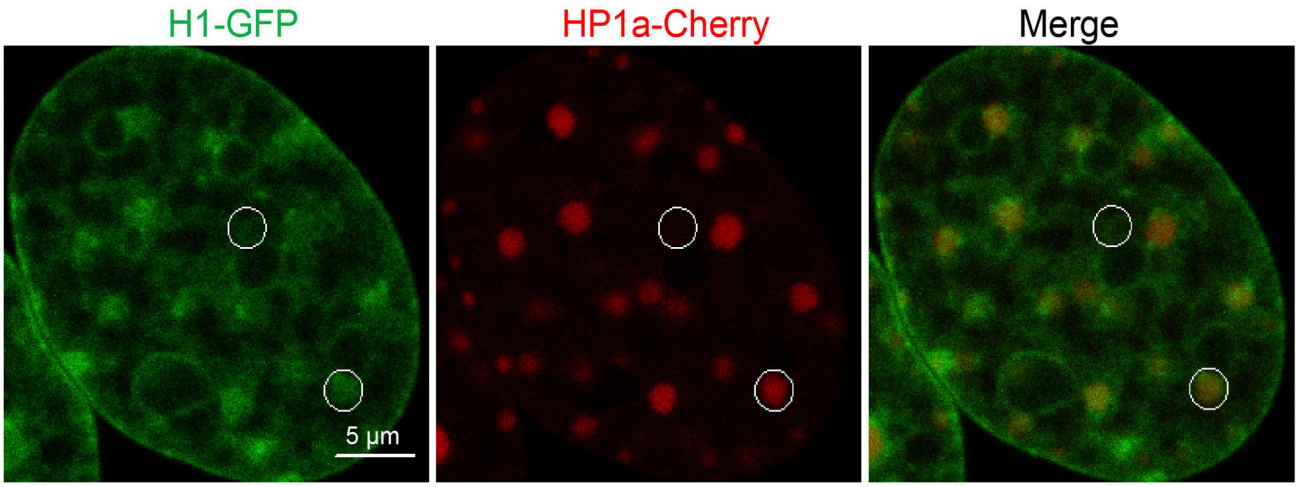

Figure 2. Region selection for FRAP

The regions marked with HP1α are selected as heterochromatin foci, while the other regions are euchromatin. Scale bar, 5 μm.

c. Choose 488 nm laser for bleaching and adjust the bleaching laser power. Click the "Test Bleach" to find the popper laser power or directly click "start experiment" to acquire a FRAP movie and evaluate the bleaching efficiency. The bleaching efficiency should be at least 70%. The setting for bleaching should not be changed in the whole experiment after finding a proper setting.
d. Open the setting bar of "Time Series", set the cycles as 200 and interval as 1.0 s.
e. Open the setting bar of "definite focus" and choose "defined" model to keep focus.

9. Adjust the setting on the microscope and make sure only acquiring a single channel image as H1-GFP (Click off the tracker for acquiring HP1α-cherry, Movie S1). Record the image series containing 5 frames before bleaching and 2–3 min time-lapse images after bleaching.

⚠ CRITICAL: Before the imaging work, replace the cell with fresh cell culture medium. The incubation system on the microscope should be settled at least 10 min before the experiments. The fraction of temperature will shift the focus on time-lapse imaging. Troubleshooting 3.

Choose the cell with proper H1-GFP signal level. Too weak the signal of H1-GFP may easily get photobleached. Troubleshooting 1.

Fluorescence intensity measurement

The image J software could be used to quantify the fluorescence intensity with raw images of FRAP acquired by confocal microscope. The times-lapse FRAP images might have some drift both from system drift or cell movement, which should be corrected. The fluorescence intensity of bleached regions could be quantified after drift correction.

10. Drift correction
    a. Open the raw images with Fiji image J (Methods video S2).
    b. Crop an interesting region containing one cell or only one nucleus.
    c. Correct the image shift and rotation with TurboReg and StackReg tool. Select the tool as following. Image J > Plugins > StackReg.

11. Find the bleached regions.

a. Find the bleached regions with the ROI manager tool. Click the selecting tool on the main panel of image J. Draw a circle with 20 pixels in diameter as follows. Analyze>ROI manager>-More>specify. Click 'oval' and set the diameter as 20 pixels, the same size as photobleaching setup.
b. Move the selecting circle to the bleached region at the fifth frame. Click 'update' on the ROI manager tool window.
c. Add a circle at the non-nuclear region as background.

12. Measure the fluorescence intensity in the selected regions.
    a. Open the Multi-measure tool as following. Plugins>Multi Measure.
    b. Select all the circles and add them to the multi-measure box using the tool of add<SP> in this window of this plugin.
    c. Click Multi-Measure on the tool window of the multi-measure plugin. The intensity of the regions will be shown in a new window.
    d. Copy the data and paste it into a new work sheet of excel (Table 1).

Optional: Image J with proper plugins for recognizing the raw images generated by the confocal microscope could also be used.

FRAP curve

13. Fluorescence intensity calculation.
    a. The fluorescence intensity of FRAP regions should subtract the background intensity at first (Methods video S3).
    b. Calculate fluorescence intensity before bleaching region with the average fluorescence intensity in the test region with the first 5 frames.
    c. Calculate the relative fluorescence intensity. Use the fluorescence intensity of regions after bleaching relative to the average fluorescence intensity of the same region in the 5 frames of images before bleaching.

14. Construct FRAP curve.
    a. Combine all the relative fluorescence intensity data from heterochromatin regions in one group, and the data from euchromatin in another group.
    b. Open the GraphPad software. Choose the "XY" model in graph construction (New table and graph>XY>Choose a graph>Connecting line only). Input the correct number of repeat value in "Y". Choose "Mean and Error" and "SEM" in the dialog. Click "Create" to start.
    c. Paste the time point in the "X" and paste all the relative fluorescence intensity data column by column. the software will calculate the mean value and SEM of the relative fluorescence intensity of heterochromatin or euchromatin automatically.
    d. Construct the FRAP curve using GraphPad Prism by click "Graphs>Date", (Figure 3). Adjust the setup of connecting line and error bar.

15. Statistical Analysis

    a. Two tailed student t-test could be used in statistical analysis with GraphPad Prism (Melcer et al., 2012).
    b. The recovery ratio at 120 s after bleaching could also be used to evaluate the percentage of the mobile fraction (Figure 3).

Optional: The FRAP curve could also be constructed with Microsoft Excel in a similar way. The mean value and SEM/SD value could be calculated on Excel. Select the mean value of relative recovery rate and construct a line graph by using "Scatter and smooth line" model (Excel>Inset>Charts>Scatter>Scatter and smooth line). The error bar could be added by using the SEM or SD value.

Table 1. Fluorescence intensity in FRAP with H1-GFP

Frame	Region1	Region2	Background
1	134.497	105.975	1.227
2	128.413	101.714	1.045
3	126.984	101.534	1.239
4	126.763	101.335	1.076
5	124.462	101.172	1.166
6	11.499	11.528	0.928
7	15.64	15.491	1.065
8	19.708	19.867	1.16
9	24.188	22.474	1.045
10	27.675	24.186	1.031
11	31.309	28.358	1.329
12	33.951	29.742	1.249
13	35.706	32.454	1.297
14	37.998	33.045	1.172
15	41.092	35.448	1.135
16	42.501	37.487	1.19
17	45.045	38.47	1.431
18	46.444	39.078	1.225
19	47.589	40.274	1.162
20	50.168	41.08	1.166
21	50.933	42.2	1.2
22	53.129	43.139	1.166
23	53.215	43.959	1.221
24	56.272	44.732	1.106
25	56.491	46.853	1.339
26	57.307	47.618	1.266
27	58.47	47.176	1.286
28	60.147	49.564	1.17
29	61.088	49.143	1.297
30	61.783	49.575	1.301
31	62.975	50.282	1.211
32	63.855	50.012	1.07
33	63.941	51.505	1.225
34	64.204	51.959	1.2
35	67.035	53.575	1.088
36	66.356	52.194	0.996
37	67.153	52.72	1.104
38	68.387	53.319	1.331
39	68.209	53.898	1.174
40	68.869	52.888	1.225
41	69.648	53.691	1.227
42	70.671	53.597	1.133
43	71.157	54.507	1.29
44	71.37	55.143	0.996
45	71.72	55.624	1.117
46	72.348	56.225	1.188
47	74.378	55.761	1.307
48	73.693	56.372	1.249
49	73.697	56.313	1.096
50	75.489	56.417	1.162
51	75.153	57.45	1.19
52	75.137	57.524	1.249
53	74.534	56.683	1.202

(*Continued on next page*)

Table 1. *Continued*

Frame	Region1	Region2	Background
54	76.072	56.278	1.342
55	77.804	56.914	1.086
56	78.828	58.863	1.182
57	77.072	57.284	1.074
58	79.407	57.859	1.16
59	78.746	59.006	1.213
60	78.409	58.857	1.149
61	78.988	58.36	1.057
62	80.487	59.513	1.415
63	80.16	58.82	1.211
64	79.984	60.748	1.102
65	81.309	58.971	1.186
66	81.307	59.423	1.143
67	81.579	59.262	1.115
68	81.016	58.18	1.376
69	81.125	59.247	1.211
70	81.648	59.329	1.166
71	83.266	60.037	1.378
72	81.403	59.902	1.315
73	82.806	59.947	1.174
74	82.223	59.896	1.204
75	83.084	59.644	1.133
76	82.755	59.961	1.209
77	84.429	60.515	1.155
78	84.421	60.808	1.198
79	84.495	61.082	1.006
80	83.546	60.56	1.004
81	84.63	60.62	0.99
82	82.575	61.184	1.108
83	84.307	61.998	1.252
84	85.685	61.329	1.215
85	84.323	62.313	1.221
86	86.491	62.08	1.211
87	85.689	63.534	1.303
88	85.961	61.176	1.172
89	85.808	62.211	1.231
90	85.239	62.712	1.231
91	85.092	63.174	1.276
92	85.998	62.27	1.108
93	87.299	61.818	1.215
94	86.644	63.061	1.225
95	87.329	62.988	1.18
96	87.18	62.687	1.401
97	86.83	63.09	1.245
98	86.761	62.742	1.258
99	88.552	63.082	1.247
100	86.963	62.738	1.135
101	89.082	64.329	1.08
102	87.018	63.851	1.02
103	88.466	62.871	1.143
104	87.057	63.865	0.992
105	87.217	64.078	1.035
106	87.822	63.571	1.157
107	87.683	63.431	1.319

(*Continued on next page*)

Table 1. *Continued*

Frame	Region1	Region2	Background
108	88.305	63.335	1.231
109	88.215	62.935	1.057
110	88.838	62.515	1.202
111	89.603	63.802	1.178
112	89.266	63.393	1.157
113	89.446	63.975	1.409
114	88.454	62.632	1.153
115	90.624	63.55	1.274
116	88.573	64.051	1.125
117	89.393	64.507	1.389
118	89.405	64.72	1.274
119	90.761	63.227	1.213
120	88.45	63.286	1.266
121	88.524	64.26	1.397
122	87.796	62.554	1.115
123	88.526	63.37	1.247

Note: Three Plugins as StackReg, TurboReg and Multi-Measure should be installed.

EXPECTED OUTCOMES

The recovery rate and ratio of H1 with FRAP could be used to evaluate the euchromatin or heterochromatin loosening effect. The higher rate of H1-GFP recovery, the higher dynamics of chromatin and contains more loosen chromatin. The high percentage of mobile fraction also could be evidence in high mobility and loosen of chromatin. Here is an example of loosen chromatin by adding VPA in somatic cell reprogramming (Figure 4).

LIMITATIONS

The HP1α marked heterochromatin foci is not much obvious in some human cells, such as human fibroblast, SK-Hep-1 and so on (Figure 5). In such case, you can use the HP1α-cherry marker to avoid choosing heterochromatin foci and only count the euchromatin dynamics.

Cells in senescence or mitosis always have an abnormal chromatin which shows high contrast in H1-GFP between different regions.

Combining with heterochromatin foci area counting with immunofluorescence, it could tell more details in chromatin dynamics, including heterochromatin transform into euchromatin.

This method can be used to detect a global change of chromatin. The dynamics in a special gene locus could also be detected combining with a special marker in live cells.

TROUBLESHOOTING

Problem 1

The H1-GFP signal is too low and the recovery curve may not be reliable (step 2).

Potential solution

High dose of virus coding H1-GFP should be used to infect the cells. Choose the cells with brighter fluorescence could also solve the problem. Use a highly sensitive PMT in confocal imaging could also benefit for collecting weak signal, such as GaAsP PMT and so on.

Problem 2

The bleaching efficiency is too low (steps 7–9).

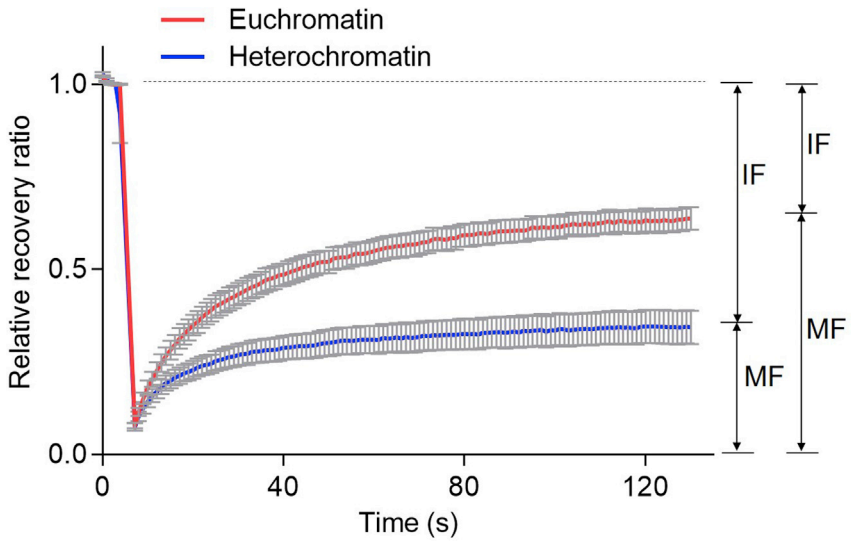

Figure 3. FRAP curve of H1-GFP in MEF cells

The mobile fraction (MF) and immobile fraction (IF) of heterochromatin and euchromatin is marked. The data were represented as mean ± SEM (n=12 for heterochromatin regions, and n=15 for euchromatin regions).

Potential solution

A high power and continues laser, such as argon laser, is better for FRAP experiment. Pulse laser may not suitable for bleaching GFP signal. Increasing the bleaching time could also increase the bleaching efficiency, but too much bleaching cycle will delay the recovery process and add more variation.

Problem 3

Focus and XY drift in time series imaging recoding in FRAP (steps 9 and 10).

Potential solution

The focus in Z-axis may be lost as temperature change or cell movement. The confocal with cells should be prewarmed for a few minutes before FRAP experiment to minimize temperature caused focus drift.

The XY drift could also be generated by cell/nucleus movement or the confocal system drift, which could be corrected by StackReg and TurboReg plugin of Image J discribed in step 10. The StackReg plugin should work together with TurboReg plugin, which could align image stack through comparing the landmarks of two neighboring images in one stack (Thevenaz et al., 1998). A focus stabilization system could also be used, such as definite focus system on Zeiss microscope or perfect focus system on Nikon microscope.

Problem 4

The photobleaching in time series imaging (steps 7–9).

Potential solution

The higher power the laser used, the more photobleaching in acquiring time series imaging. To avoid photobleaching in image acquiring, the power of laser should be as low as possible. The highly sensitive PMT would benefit the process as lower power of laser is needed for imaging. What's more, the photobleaching could be corrected by choosing a similar region in a none bleaching nucleus as reference, although it is not always available as the small imaging scale.

Problem 5

The recording time for each frame is too long (steps 8 and 9).

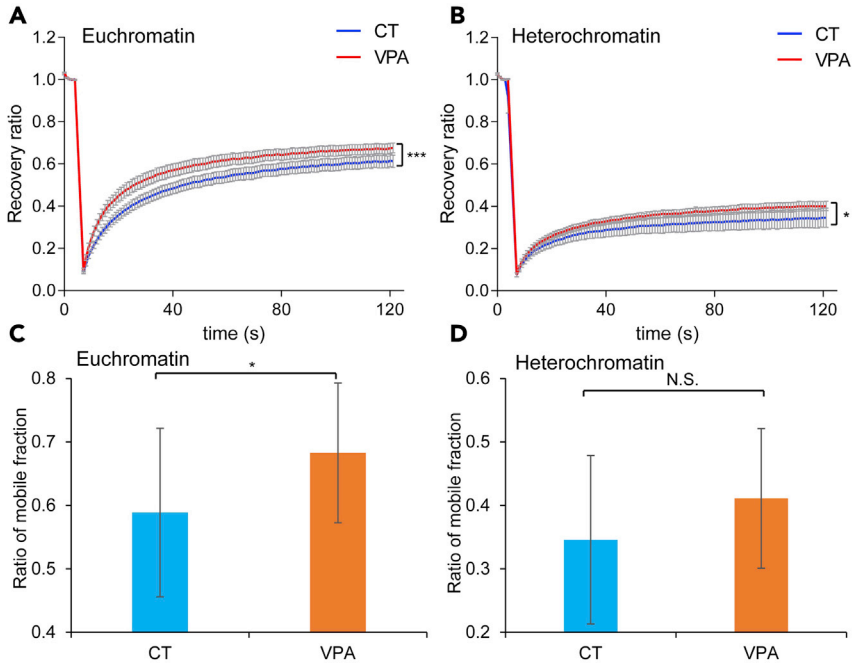

Figure 4. VPA opens chromatin

FRAP on H1-GFP were carried out with or without 200 μM VPA treatment for 48 h. HP1α-cherry is added as a maker to select either heterochromatin or euchromatin.

(A and B) The FRAP curve of euchromatin (A) or heterochromatin (B) with or without VPA.

(C and D) The ratio of MF in euchromatin (C) or heterochromatin (D).

The data were represented as mean ± SEM in A and B, while mean ± SD in C and D ($n \geq 12$ regions for each group), Two-tailed unpaired student *t*-test were used. N. S represents $p>0.05$. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

Potential solution

It should take no more than 1 s in recoding each frame image in FRAP as the recovery rate is high for H1-GFP. Too long the recoding time will lower the resolution in FRAP analysis. The resolution of each frame could be set as 512×512 to balance the contradiction between resolution and recoding rate. Choosing the round trip scan model could also reduce the recoding time, but we didn't recommend as the drift in each scan in some cases.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xingguo Liu (liu_xingguo@gibh.ac.cn).

Materials availability

All materials are available commercially. The plasmids of pMX-H1-GFP and pMX-HP1α-Cherry can be obtained through contacting the lead contact.

Data and code availability

All plugins for image J used in this study are list in the key resources table and available on NIH (https://imagej.nih.gov/ij/plugins/). Software is listed in the key resources table.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100706.

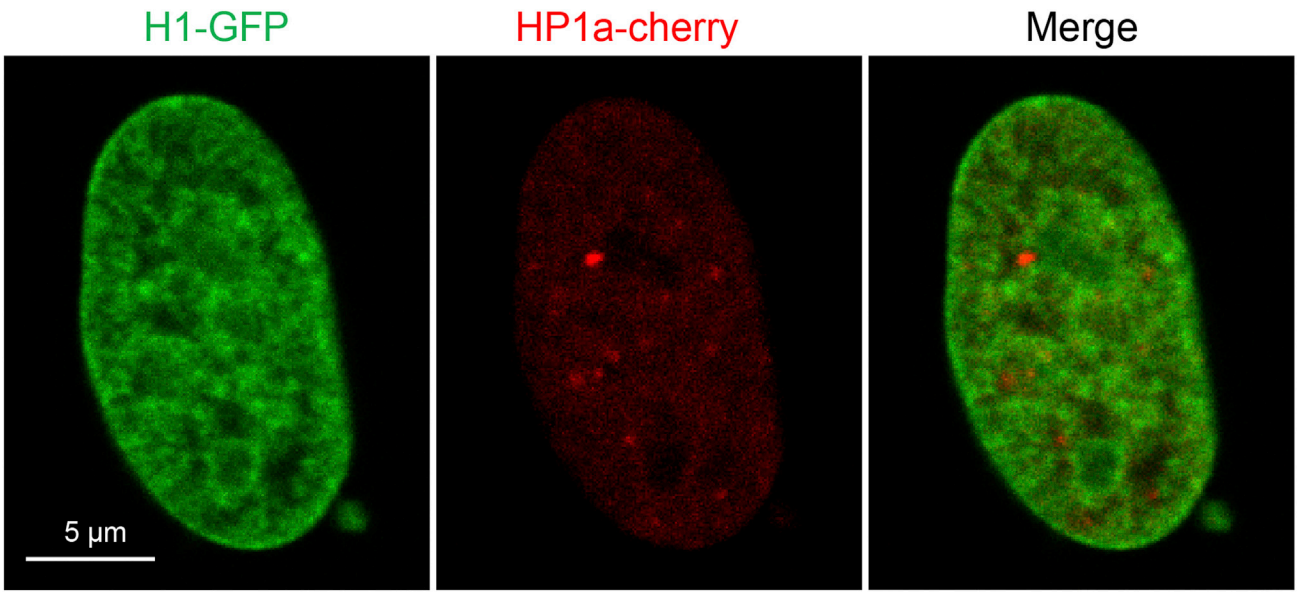

Figure 5. Chromatin foci in human fibroblast cells

Human fibroblast is labeled with H1-GFP and HP1α-cherry. HP1α-cherry is used to indicate heterochromatin foci. Scale bar, 5 μm

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AUTHOR CONTRIBUTIONS

X.L. supervised the project and wrote the manuscript. Q.L. developed the protocol. Q.L., J.Q., and W.L. completed the figures and manuscript. H.W., Y.Z., and K.C. were involved in manuscript preparation.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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