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.
Protocol

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#SLHVR335B |

| **Recombinant DNA** | | |
| pMXs-H1-GFP | This study | N/A |
| pMXs-HP1a-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 CO₂ 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, SPS; Nikon A1 or Olympus FV1200 and so on.
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 knockdown 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.

### 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.
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% CO2 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).
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.
c. Choose 488 nm laser for bleaching and adjust the bleaching laser power. Click the “Test Bleach” to find the proper 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.

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.
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>Insert>Charts>Scatter>Scatter and smooth line). The error bar could be added by using the SEM or SD value.
| Frame | Region 1 | Region 2 | 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)
| 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)
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).

| 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      |
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 recording 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 described 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).
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-HP1x-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.
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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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