Supplementary Note: Chromatin immunoprecipitation protocols

ChIPmentation was tested and validated in combination with three different chromatin immunoprecipitation (ChIP) protocols that are described below.

ChIP version 1 (used for H3K4me3 and H3K27me3)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1 ml PBS for 5 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 μM PMSF. The pellet was lysed in Cell Lysis Buffer (50 mM HEPES/KOH pH 7.4, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10% Glycerol, 0.5% NP-40, 0.25% Triton X-100, 1x protease inhibitors (Sigma)) for 10 minutes on ice. Nuclei were isolated by spinning the lysed cells for 10 minutes at 1,000 x g at 4°C, the supernatant was discarded, and the pellet was resuspended in Sonication Buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.1% SDS) and sonicated in a 130 μl microTUBE (for up to 3 x 10^6 cells) on a Covaris S220 for 12 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 2%, peak incident power 105 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C and the supernatant was transferred to a new tube. The lysate was adjusted to 200 μl per IP with a buffer composition of 20 mM HEPES, 0.1% SDS, 1%Triton X-100, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and incubated with an antibody against H3K4me3 (1 μg/IP, Diagenode pAb-003-050) or H3K27me3 (1 μg/IP, Diagenode pAb-195-050) overnight at 4°C on a rotator. 20 μl of Protein A (or Protein G, dependent on the antibody used) magnetic beads were blocked overnight with 0.1% BSA in PBS and added to the IP the next day for 2 hours on a rotator at 4°C to capture the immunoprecipitated fragments. The immunoprecipitated chromatin was washed subsequently with WBI (20 mM HEPES, 150 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (twice), WBII (20 mM HEPES, 500 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (once), WBIII (20 mM HEPES, 250 mM LiCl, 0.5% DOC, 0.5% NP-40, 1 mM EDTA, 0.5 mM EGTA) (once), and WBIV (20 mM HEPES, 1 mM EDTA, 0.5 mM EGTA) (twice). Beads were then incubated with 70 μl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 μl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 μl of elution buffer was added to the beads for 1 minute, and eluates were combined and incubated with another 1 μl of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 2 (used for H3K4me1, H3K36me3, and REST)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 μM PMSF. The pellet was lysed in RIPA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 140 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC, 1x protease inhibitors (Sigma)) and sonicated in a 1 ml milliTUBE in a Covaris S220 for 30 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, 50 μl (10 μl for low-input ChIPmentation) magnetic Protein A or Protein G beads (dependent on the antibody used) were blocked and conjugated to an antibody by washing and resuspending twice in PBS, 0.5% BSA, 0.5% Tween-20. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K4me1 (1 μg/IP, Diagenode pAb-194-050), H3K36me3 (1 μg/IP, Diagenode pAb-192-050), and REST (10 μg/IP, Millipore 07-579). Blocked antibody-conjugated beads were then placed on a magnet, supernatant was removed, and the sonicated lysate was added to the beads followed by incubation for 3 hours at 4°C on a rotator. Beads were washed subsequently with 150 μl RIPA (twice), RIPA-500 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 500 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC,) (twice), RIPA-LiCl (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 250 mM LiCl, 1% Triton X-100, 0.5% DOC, 0.5% NP40), and TE pH 8.0 (twice). Beads were then incubated with 70 μl elution buffer (0.5% SDS, 300 mM

NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 μl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 3 (used for H3K27ac, PU.1, CTCF, and GATA1)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 5-10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 μM PMSF. The pellet was lysed in buffer L3B (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, 1x protease inhibitors (Sigma)) and sonicated in a 1ml milliTUBE in a Covaris S220 for 20 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were supplemented with 1% Triton-X-100 and centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, beads were blocked and conjugated to an antibody by washing them twice in PBS with 0.5% BSA and resuspending 50 μl (10 μl beads for low-input ChIPmentation) of magnetic Protein A or Protein G beads (dependent on the antibody used) per IP in 200 µl of PBS with 0.5% BSA. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K27ac (2 μg, Diagenode pAb-196-050), PU.1 (5 μg/IP, Santa Cruz sc-352), CTCF (10 μl/IP, Millipore 07-729), and GATA1 (4 μg/IP and 2 μg for low-input, Abcam ab11852). Blocked antibody conjugated magnetic beads were added to the tube containing the chromatin and incubated for 3 hours at 4°C. Beads were washed subsequently with 150 μl TF-WBI (20 mM Tris-HCl/pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA) (twice), TF-WBIII (250 mM LiCl, 1% Triton X-100, 0.7% DOC, 10 mM Tris-HCl, 1 mM EDTA) (twice), and TET (0.2% Tween-20, 10 mM Tris-HCl/pH 8.0, 1 mM EDTA) (twice). Beads were then incubated with 70 μl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris HCl pH 8.0) containing 2 μl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 μl of elution buffer was added to the beads for 1 minute and eluates were combined and incubated with another 1 μl of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.