Dilution Hi-C

Dilution Hi-C was performed as in Lieberman-Aiden et al. (2009)

Hi-C method

Crosslinking of cells. Human cell line GM06990, an EBV-transformed lymphoblastoid cell line (Coriell, Camden, NJ), was cultured in RPMI1640, 15% fetal calf serum, 1% penicillin-streptomycin, and 2mM L-glutamine. Human erythroleukemia cell line K562 (ATCC, Manassas, VA) was cultured in DMEM, 10% fetal calf serum, 1% penicillinstreptomycin, and 2mM L-glutamine. One hundred million cells were spun down and resuspended in 45 ml fresh medium. Cells were fixed by adding 1.25 ml 37% formaldehyde and incubating for 10 minutes at room temperature (RT). The reaction was stopped by adding 2.5 ml 2.5 M glycine. The cell suspension was incubated for 5 minutes at RT, followed by 15 minutes on ice. The crosslinked cell suspension was split into 4 equal parts and centrifuged at 1500 rpm for 10 minutes. The supernatant was discarded and the cell pellets were stored at -80C.

Cell lysis and chromatin digestion. For cell lysis, 550 µl lysis buffer (500 µl 10 mM Tris-HCl pH8.0, 10 mM NaCl, 0.2% Ige cal CA630; 50 µl protease inhibitors (Sigma, St. Louis, MO) were added to one batch of cells (~ 25 million cells). Cells were incubated on ice for at least 15 minutes. Next, cells were lysed with a Dounce homogenizer by moving the pestle A up and down 10 times, incubating on ice for one minute followed by 10 more strokes with the pestle. The suspension was spun down for 5 minutes at 5000 rpm at RT. The supernatant was discarded and the pellet was washed twice with 500 µl icecold 1x NEBuffer 2 (NEB, Ipswich, MA). The pellet was then resuspended in 1x NEBuffer 2 in a total volume of 250 µl and split into five 50 µl aliquots. Next, 312 µl 1x NEBuffer 2 was added per tube. To remove the proteins that were not directly crosslinked to the DNA, 38 µl 1% SDS was added per tube and the mixture was resuspended and incubated at 65°C for 10 minutes exactly. Tubes were put on ice and 44 µl 10% Triton X-100 was added and mixed carefully avoiding bubbles to quench the SDS. Chromatin was subsequently digested overnight at 37°C by adding 400 Units HindIII (NEB).

Marking of DNA ends and blunt-end ligation. Five tubes with digested chromatin were put on ice and tube 1 was kept separate and served as a 3C control. To fill in and mark the DNA ends, 1.5 µl 10 mM dATP, 1.5 µl 10 mM dGTP, 1.5 µl 10 mM dTTP, 37.5 µl 0.4 mM biotin-14-dCTP (Invitrogen, Carlsbad, CA) and 10 µl 5U/µl Klenow (NEB) were added to tubes 2-5. The mixtures were incubated at 37°C for 45 minutes and subsequently placed on ice. Enzymes were inactivated by adding 86 µl 10% SDS to tubes 1-5 and incubating all tubes at 65°C for 30 minutes. Tubes were placed on ice immediately. Five 15 ml tubes were prepared, each containing 7.61 ml ligation mix (745 µl 10% Triton X-100, 745 µl 10x ligation buffer (500 mM Tris-HCl pH7.5, 100 mM MgCl2, 100 mM DTT), 80 µl 10 mg/ml BSA, 80 µl 100 mM ATP and 5.96 ml water). Each digested chromatin mixture was transferred to a corresponding 15 ml tube. For normal 3C ligation 10 µl 1U/µl T4 DNA ligase (Invitrogen) was added to tube 1. For blunt-end ligation 50 µl 1U/µl T4 DNA ligase was added to tubes 2-5. All 5 tubes were incubated at 16°C for 4 hours.

DNA purification. To reverse crosslinks and to degrade protein, 50 µl 10 mg/ml proteinase K was added per tube and the tubes were incubated overnight at 65°C. The next day an additional 50 µl 10 mg/ml proteinase K was added per tube and the incubation was continued at 65°C for another 2 hours. Reaction mixtures were cooled to RT and transferred to five 50 ml conical tubes. The DNA was extracted by adding 10 ml phenol pH8.0, vortexing for 2 minutes and spinning for 10 minutes at 3,500 rpm. The supernatants were transferred to five new 50 ml conical tubes. Another DNA extraction

was performed with 10 ml phenol pH8.0:chloroform (1:1). After vortexing and centrifugation for 10 minutes at 3,500 rpm, the supernatants were transferred to five 35 ml centrifugation tubes. The volume was brought to 10 ml per tube with 10 mM Tris pH8.0, 1 mM EDTA (1x TE). To precipitate the DNA, 1 ml 3M Na-acetate was added per tube and mixed well before adding 25 ml ice-cold 100% ethanol. Tubes were inverted several times to properly mix the contents and were incubated at -80°C for at least one hour. Next, the tubes were spun at 4°C for 20 minutes at 10,000xg. The supernatant was discarded and each DNA pellet was dissolved in 450 µl 1x TE and transferred to a 1.7 ml centrifuge tube. The DNA was extracted twice by adding 500 µl phenol pH8.0:chloroform (1:1), vortexing for 30 seconds and spinning at 14,000 rpm for 5 minutes at RT. After the second extraction, the supernatants (each ~400 µl) were transferred to five new 1.7 ml tubes and 40 µl 3M Na-acetate was added per tube and mixed. Next, 1 ml 100% ethanol per tube was added. After inverting the tubes several times, the tubes were incubated at - 80°C for at least 30 minutes. Tubes were spun at 18,000xg for 20 minutes at 4°C. The supernatant was discarded and the pellets were washed once with 500 µl 70% ethanol. After centrifuging at 14,000 rpm for 5 minutes, the supernatant was discarded and the pellets were air-dried briefly prior to resuspending in 25 µl 1x TE. To degrade any purified RNA, 1 µl 1 mg/ml RNAse A was added per tube and incubated at 37°C for 15 minutes. The Hi-C contents of tubes 2-5 were pooled and tube 1 was kept separate as the 3C control.

Quality control HiC libraries. Both 3C and Hi-C libraries were checked for quality and quantified by running an aliquot on a 0.8% agarose gel. To confirm that the ligation process worked as intended, we used the fact that successful fill-in and ligation of HindIII sites (AAGCTT) should create sites for the restriction enzyme NheI (GCTAGC). We used PCR to amplify a ligation product formed from two nearby restriction fragments and determined that 70% of amplicons were cut only by NheI (Fig. S7). Sequences of the primers used for checking libraries are:

HindIII -1 GTTCATCTTGCTGCCAGAAATGCCGAGCCTG
HindIII-2 ATCCCAGCTGTCTGTAGCTTTAGAAAGTGGG
NcoI-1 ACCTGTTGTTTAATGAAGGGGCTCAGAAGC
NcoI-2 GTTTGCAGTGTGCTGTGCAGCATGTGTGTA

Removal of biotin from unligated ends. Biotin-14-dCTP at non-ligated DNA ends was removed with the exonuclease activity of T4 DNA polymerase. To this end 5 µg of Hi-C library was added to 1 µl 10 mg/ml BSA, 10 µl 10x NEBuffer 2, 1 µl 10 mM dATP, 1 µl 10 mM dGTP and 5 Units T4 DNA polymerase (NEB) in a total volume of 100 µl and incubated at 12°C for 2 hours. If possible, multiple 5 µg reactions were performed. Reactions were stopped by adding 2 µl 0.5 M EDTA pH8.0. DNA was subsequently purified with one phenol pH8.0:chloroform (1:1) extraction followed by ethanol precipitation. DNA pellets were resuspended and pooled in a total of 100 µl water. Shearing and size selection. The DNA was sheared to a size of 300-500 basepairs with a Covaris S2 instrument (Covaris, Woburn, MA), Duty cycle 5, Intensity 5, Cycles/burst 200, time 60 secs for 4 cycles. The DNA ends were repaired by adding 14 µl 10x ligation buffer (NEB), 14 µl 2.5 mM dNTP mix, 5 µl T4 DNA polymerase (NEB), 5 µl T4 polynucleotide kinase (NEB), 1 µl Klenow DNA polymerase (NEB) and 1 µl water and was incubated at 20oC for 30 minutes followed by purification of the DNA with a Qiagen MinElute column (Qiagen, Valencia, CA). The DNA was eluted with 2x 15 µl 10 mM Tris pH8.0, 0.1 mM EDTA. Next, an 'A' was added to the 3' ends of the end repaired DNA by addition of 5 µl 10x NEBuffer2, 10 µl 1 mM dATP, 2 µl water and 3 µl Klenow (exo-) (NEB) . The reaction was incubated at 37°C for 30 minutes followed by 65°C for 20 minutes to inactivate Klenow (exo-). The reactions were cooled on ice and the volume was reduced to 20 µl with a speedvac. DNA was electrophoresed on a 1.5% agarose gel

in 1X TAE for 3.5 hours at 80 V. The gel was stained with SYBR green (Lonza Walkersville, Basel, Switzerland), visualized on a DarkReader (Clare Chemical, Dolores, CO) and DNA between 300 and 500 base pairs was excised and purified with a gel extraction kit (Qiagen). The gel slices were solubilized with three volumes of Buffer QG (Qiagen) at RT and the DNA purified with QIAquick spin columns (Qiagen). The DNA was eluted twice with 50 µl 10mM Tris pH 8.0, 0.1 mM EDTA and the final volume was made up to 300 µl with 10 mM Tris pH 8.0, 0.1 mM EDTA. The DNA concentration was measured with the Quant-iT assay (Invitrogen).

Biotin pull-down and Paired End sequencing. All subsequent steps were performed in DNA LoBind tubes (Eppendorf, Westbury, NY). The biotin tagged Hi-C DNA was bound to Dynabeads MyOne Streptavin C1 Beads (Invitrogen) as follows. Sixty µl of resuspended Streptavidin beads were washed twice with 400 µl Tween Wash Buffer (TWB) (5 mM Tris-HCl pH8.0, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween) by incubating for 3 minutes at RT with rotation. After this and for all subsequent incubations or washes of Streptavidin beads, the beads were reclaimed by holding against a magnetic particle concentrator (Invitrogen) for 1 minute and the supernatant was removed. These reclaimed beads were then resuspended in 300 µl 2x Binding Buffer (BB) (10 mM TrisHCl pH8.0, 1 mM EDTA, 2 M NaCl) and combined with 300 µl Hi-C DNA. The mixture was incubated at RT for 15 minutes with rotation. The supernatant was removed and the DNA bound Streptavidin beads were resuspended in 400 µl 1x BB and transferred to a new tube. The beads were then resuspended in 100 µl 1x ligation buffer, transferred to a new tube before a final resuspension in 50 µl 1x ligation buffer. Six picomoles of Illumina Paired End adapters (Illumina, San Diego, CA) per µg of Hi-C DNA (measured after Qiagen gel purification) were ligated to the Hi-C DNA for 2 hours at RT in the presence of, 1 mM ATP and 20U T4 DNA Ligase (Ambion, Austin, TX). The ligated HiC DNA was isolated by holding against the magnet and was washed with 400 µl of 1x TWB to remove non-ligated Paired End adapters. The beads were resuspended in a further 400 µl 1x TWB and the mixture was transferred to a new tube and the Streptavidin beads were recovered. This wash step was repeated with 200 µl 1x BB, then 200 µl 1x NEBuffer 2 and finally 50 µl 1x NEBuffer 2. The beads were resuspended in 50 µl 1x NEBuffer 2. Next, test PCR reactions were performed to determine the optimal PCR cycles needed to generate enough library for sequencing. Four trial PCR reactions, each containing 0.6 µl Streptavidin bead bound Hi-C library and Illumina PE1.0 and PE2.0 PCR primers (1.5 pmol each) in 10 µl 1x Phusion High Fidelity master mix with HF buffer (NEB), were set up to determine the number of cycles necessary to generate enough PCR product for sequencing. The temperature profile was 30 s at 98°C followed by 9, 12, 15 or 18 cycles of 10 s at 98°C, 30 s at 65°C, 30 s at 72°C and a final 7-minute extension at 72°C. The PCR reactions were run on a 5% polyacrylamide gel, stained with Sybr Green and the optimal cycle number was determined. A large-scale PCR was then set-up with the remainder of the Streptavidin bead bound Hi-C library with the number of PCR cycles determined by the trial PCR. 1% of the large scale PCR product was kept to run on a gel. The PCR product was purified by mixing with 1.8x volume Ampure beads (Beckman Coulter, Fullerton, CA). The mix was held against a magnet to separate the PCR product bound to the Ampure beads and the supernatant was discarded. The Hi- C library bound Ampure beads were washed twice with 1 ml 70% ethanol while the tube remained against the magnet. After air-drying the beads, the DNA was eluted by resuspending the beads in 50 µl of 10 mM Tris pH8.0, 0.1 mM EDTA. The tube was held against a magnet and the supernatant containing the purified PCR products was transferred to a new tube. Next, 1% of the Ampure bead purified PCR product was compared against the 1% aliquot of original PCR product on a 5% polyacrylamide gel. Finally, the Hi-C library was sequenced with Illumina paired end sequencing.