**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.