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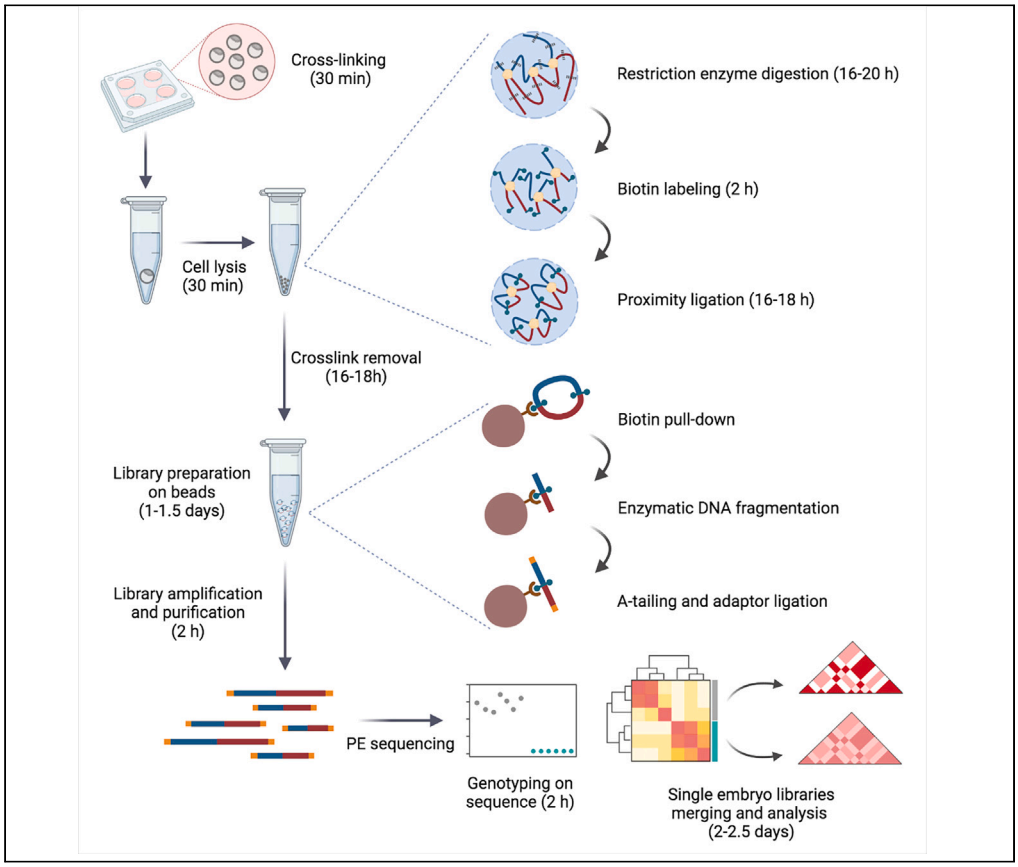
Protocol

A protocol to study three-dimensional genome structure in individual mutant preimplantation mouse embryos



Hi-C studies the three-dimensional structure of the genome by detecting genome-wide chromatin regions that are in spatial proximity within the nucleus. We developed single-blastocyst Hi-C in mutant mouse embryos to genotype them on sequence. We describe steps for embryo fixation and nuclei permeabilization, after which chromatin is digested and re-ligated having incorporated a biotin-labeled nucleotide at the ligation junction. After cross-link reversal, we then detail purification of immobilized chimeric DNA ligations, library generation, sequencing, and genome-wide analysis of interactions.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol to study the 3D organization of chromatin in preimplantation mouse embryos

Steps for performing single-embryo Hi-C and library preparation

Single-mutant embryo Hi-C library genotyping on sequence

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A protocol to study three-dimensional genome structure in individual mutant preimplantation mouse embryos

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SUMMARY

Hi-C studies the three-dimensional structure of the genome by detecting genome-wide chromatin regions that are in spatial proximity within the nucleus. We developed single-blastocyst Hi-C in mutant mouse embryos to genotype them on sequence. We describe steps for embryo fixation and nuclei permeabilization, after which chromatin is digested and re-ligated having incorporated a biotin-labeled nucleotide at the ligation junction. After cross-link reversal, we then detail purification of immobilized chimeric DNA ligations, library generation, sequencing, and genome-wide analysis of interactions.

For complete details on the use and execution of this protocol, please refer to Andreu et al. (2022).[1]

BEFORE YOU BEGIN

This protocol describes the steps for performing Hi-C in single mouse blastocysts. This single embryo approach is necessary when mutant embryos are phenotypically indistinguishable from littermates, as in our case for *Ctcf* mutant blastocysts. Individual embryo Hi-C libraries are generated to afterward genotype them on the sequence data, and finally merge data from individual Hi-C libraries by genotype for posterior analysis. To optimize the number of libraries generated, we used embryos from the cross of $Ctcf^{fl/-}$; $Zp3\text{-}Cre^{tg/+}$ females with $Ctcf^{fl/-}$ males, which produce 50% full mutant embryos. We also included control embryos (CD1) in the study. We set up the protocol for blastocyst stage but it can be easily adjusted for other preimplantation stages of mouse development.

Institutional permissions

Mice were housed and maintained in the animal facility at the Centro Nacional de Investigaciones Cardiovasculares (CNIC, Madrid, Spain) in accordance with national and European legislation. Procedures were approved by the CNIC Animal Welfare Ethics Committee and by the Area of Animal Protection of the Regional Government of Madrid (ref. PROEX 196/14). All experiments conducted using animals require permissions and approval from the relevant institutions.

Embryo collection

Timing: 5–7 days

1. Set up the necessary matings to obtain the blastocysts.
2. Dissect the uterus and flush the embryos out using M2 medium.

Preparation of solutions

Timing: 10 min

3. Prepare fresh lysis buffer on advance and keep it on ice.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Acid Tyrode's solution	Sigma-Aldrich	Cat# T-1788
M2 medium	Sigma-Aldrich	Cat# M7167-100ML
16% Formaldehyde (w/v), methanol free	Thermo Fisher	Cat# 28906
Complete protease inhibitor, EDTA free	Roche	Cat# 1187358001
MboI	New England Biolabs	Cat# R0147M
Biotin-14-dATP	Thermo Fisher	Cat# 19524-016
DNA Polymerase I, large (Klenow)	New England Biolabs	Cat# M0210L
dNTP set (100 mM each) 11969064001	Sigma	Cat# 11969064001
T4 DNA Ligase (2000 U/mL)	New England Biolabs	Cat# M0202M
Dynabeads MyOne Streptavidin C1	Thermo Fisher	Cat# 65001
AluI	New England Biolabs	Cat# R0137S
T4 polynucleotides kinase	New England Biolabs	Cat# M0201L
T4 DNA polymerase	New England Biolabs	Cat# M0203L
Klenow Fragment 3'->5' exo-	New England Biolabs	Cat# M0212S
Quick Ligation Buffer 5x	New England Biolabs	Cat# B6058S
Proteinase K	Roche	Cat# 10910000
RNAse Dnase-free	Sigma	Cat# 11119915001
Agencourt AMPure XP Beads	Beckman Coulter	Cat# A63880
NEBNext® High-Fidelity 2X PCR Master Mix	New England Biolabs	Cat# M0541S
Critical commercial assays		
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1)	New England Biolabs	Cat# E7335S
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 2)	New England Biolabs	Cat# E7500S
NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 3)	New England Biolabs	Cat# E7710S
Qubit™ dsDNA HS Assay Kit (100 assays)	Thermo Fisher	Cat# Q32851
Experimental models: Organisms/strains		
CD-1 mice	Charles Rivers	Crl:CD1(ICR)
Ctcf floxed allele mice	Heath et al.[2]	NA
Zp3-Cre$^{tg/tg}$ mice	Lewandoski et al.[3]	NA
Software and algorithms		
TADbit	Serra et al.[4]	https://github.com/3DGenomes/tadbit
Other		
Steritop Quick Release	Millipore	Cat# S2GPT05RE
Millex-GV Syringe Filter Unit, 0.22 µm, PVDF, 33 mm, gamma sterilized	Millipore	Cat# SLGV033RS
Microcapillary mouth pipette	Sigma	Cat# A5177-5EA
Holding micropipette EZ-Strip	RI	Cat# 7-72-1131/20
35 mm TC-treated Easy-Grip Style Cell Culture Dish	Falcon	Cat# 353001
4-Well dishes	Thermo Fisher	Cat# 144444
DNA LoBind 1.5mL microcentrifuge tubes	Sigma	Cat# Z666548
Qubit™ Assay Tubes	Thermo Fisher	Cat# Q32856
DynaMag-2 magnetic stand	Thermo Fisher	Cat# 12321D

MATERIALS AND EQUIPMENT

Alternatives: This protocol uses enzymatic digestion with AluI[5] instead of sonication before library generation on magnetic beads to limit sample loss, as we are working with extremely low input samples (40–60 cells). However, another method suitable for limiting starting material is DNA fragmentation with Tn5-transposase.[6] Tn5-transposase cuts DNA and introduces barcodes in one-step, what reduces workflow steps and hands-on time.

Alternatives: This protocol uses a Bioanalyzer (Agilent) to assess the range of DNA fragmentation but equivalent instruments as LabChip (PerkinElmer) can be used. All solutions indicated as filtered were filter-sterilized using vacuum or syringe filters depending on the total volume.

Lysis buffer

Reagent	Final concentration	Amount
1 M Tris-HCl pH8.0 (filtered)	10 mM	50 µL
5 M NaCl	10 mM	10 µL
10% Igepal CA-630 (NP-40)	0.2%	100 µL
100X Protease inhibitors	1X	50 µL
ddH_2O nuclease-free		4.79 mL
Total		**5 mL**

Prepare fresh 10% Igepal CA-630 (NP-40) and lysis buffer every time. Keep lysis buffer on ice.

Binding buffer 2X (2X BB)

Reagent	Final concentration	Amount
1 M Tris-HCl pH7.5 (filtered)	10 mM	60 µL
0.5 M EDTA	1 mM	12 µL
5 M NaCl (filtered)	2 M	2.4 mL
ddH_2O nuclease-free		3.528 mL
Total		**6 mL**

Prepare fresh Binding buffer 2X every time and keep it at 4°C.

Other solutions

Solution	Reagents
2% Formaldehyde	62,5 µL of Formaldehyde 16% methanol free and 437,5 µL of 1X PBS. Prepare it fresh just before using it.
100X Protease inhibitors cocktail	One tablet in 500 µL of nuclease-free ddH_2O. Store in aliquots for 6–12 months at −20°C.
1X Binding buffer (1X BB)	5 mL of 2X BB and 5 mL nuclease-free ddH_2O. Keep at 4°C for one week.
1X BB + 0.05% Tween 20	5 µL of 10% Tween 20 and 1 mL of 1X BB. Prepare it fresh and keep at 4°C.
Elution buffer (EB)	10 mM Tris-HCl pH7.5. Keep at 20°C–25°C for one week.
2 M Glycine	60 g glycine in 1 L of nuclease-free ddH_2O. Store at 20°C–25°C for one year.

STEP-BY-STEP METHOD DETAILS

Zona pellucida removal and embryo fixation

Timing: 30 min

Removing the *zona pellucida* of preimplantation embryos facilitates cell lysis and nuclei permeabilization to the different enzymes.

1. Treat the embryos with Tyrode acidic solution for 1–2 min to remove the *zona pellucida*.

   Note: Changing the embryos from drop to drop of 50 μL of Tyrode acidic solution on a 35mm dish makes the process quicker. In this part of the protocol, a mouth or hand micropipette should be used for preimplantation embryos handling and it is necessary to work under a stereomicroscope.

2. Wash the embryos three times with M2 medium by changing them from drop to drop of 30 μL on a 35mm dish.
3. Fix the embryos in 500 μL of 2% formaldehyde in a 4-well dish for 10 min at 20°C–25°C.

   Note: Formaldehyde is toxic and it should be handled in a fume hood and discarded according to local regulations. Around 20–30 blastocysts can be fixed in the same well.

4. Add 30 μL of Glycine solution 2 M (final concentration of 0.120 M Glycine) to quench the cross-linking and incubate for 10 min on ice.

   Note: Store 2 M Glycine solution at 20°C–25°C for one year.

5. Wash briefly the embryos in 500 μL of PBS 1X in a 4-well dish and then transfer each embryo individually into a clean microcentrifuge tube.

   Note: When transfering the embryos try to use the smallest possible volume.

   ⚠ CRITICAL: From now on use only DNA low-binding tubes.

Cell lysis and restriction enzyme digestion

Timing: 16–20 h

The following steps prepare the nuclei for chromatin digestion.

6. Incubate the embryos on ice in 100 μL of freshly prepared ice-cold lysis buffer for 30 min with occasional mixing by tapping.
7. Centrifuge 5 min at 900 g at 4°C, discard the supernatant carefully with a hand pipette, briefly wash the single embryo with 100 μL of cold 1X Cutsmart (or 1X NEBuffer2) and centrifuge again.

   ⚠ CRITICAL: The individual embryos cannot be seen by eye, be extremely careful when discarding the supernatant.

8. Centrifuge 5 min at 900 g at 4°C, discard the supernatant carefully and add 20 μL of 0.5% SDS in 1X Cutsmart or 1X NEBuffer2 (0.5 μL of 20% SDS (v/v) in 19.5 μL of 1X Cutsmart or 1X NEBuffer2). Incubate at 65°C for 10 min.

   ⚠ CRITICAL: The individual embryos cannot be seen by eye, be extremely careful when discarding the supernatant.

9. Place the tubes on ice and add 40 μL of 1X Cutsmart (or 1X NEBuffer2) and 12 μL of 10% Triton X-100 to quench the SDS (final concentration of 1.7% Triton X-100). Incubate at 37°C for 15 min.
10. Add 125 U of MboI and incubate for 16–18 h at 37°C in constant agitation in a microcentrifuge tube thermomixer.

    Optional: Next morning, add 50 U of MboI and incubate for additional 2 h at 37°C in constant agitation.

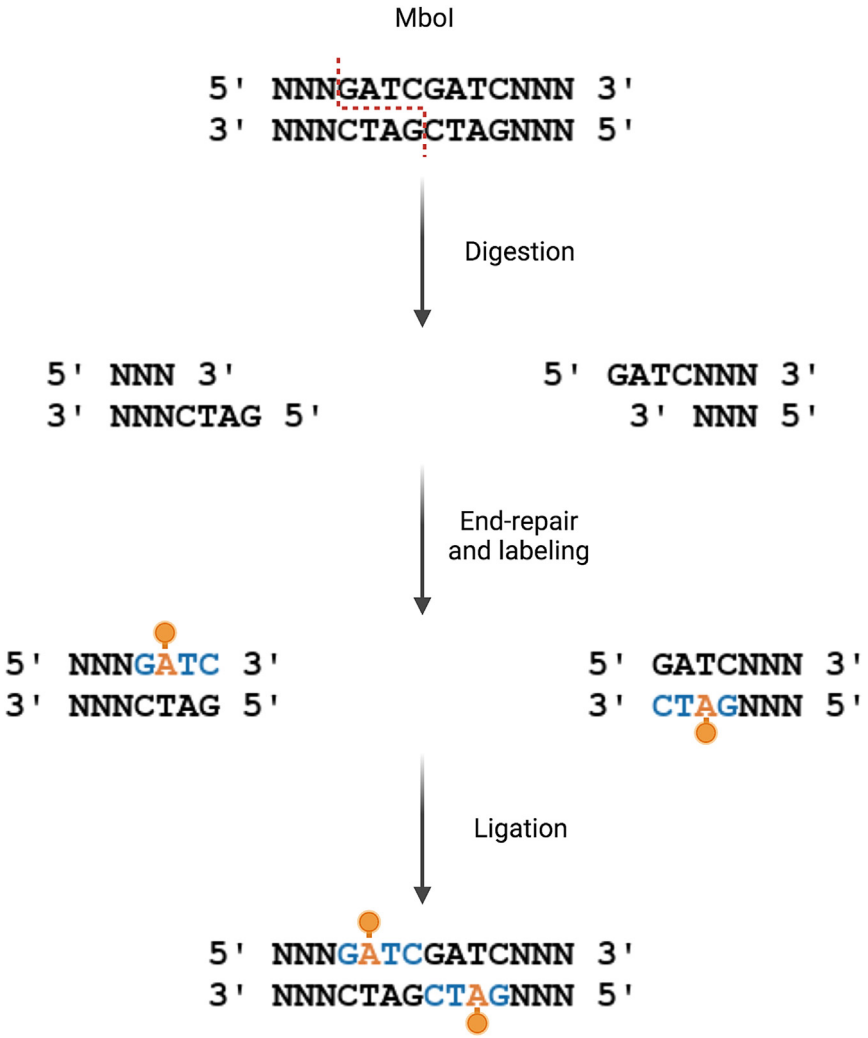

Figure 1. End-repair and labeling strategy

Digestion by MboI generates 5′ overhangs (5′ GATC 3′) that are filled in by the Klenow fragment of DNA polymerase I in presence of dNTPs to produce blunt ends. For biotin labeling dATP is replaced by biotinylated ATP (Biotin-14-dATP) in the end-repair reaction. In this case, Biotin-14-dCTP is not recommended as the labeling of the last positions of the overhang could interfere with the posterior ligation of the blunt ends.

End-repair and labeling

Timing: 2 h

Biotin-labeling of the overhang ends before ligation permits the selection of the informative DNA fragments before library generation.

11. Add 10 μL of repair and labeling mix to the same tubes from step 10 containing the single embryo samples and incubate at 37°C for 90 min with occasional mixing by tapping.

Note: The biotinylated nucleotide used in the repair and labeling mix should be compatible with the sequence of the overhang generated by the restriction enzyme used in the digestion step. Labeling of the last positions of the overhang is not recommended since it could interfere with the ligation (Figure 1).

Repair and labeling mix	
Reagent	Amount
10 mM dCTP	0.3 μL
10 mM dGTP	0.3 μL
10 mM dTTP	0.3 μL
0.4 mM Biotin-14-dATP	7.5 μL
DNA Polymerase I Large (Klenow) 5 U/μL	1.6 μL

12. Incubate at 65°C for 15 min to inactivate the enzyme.

In-nuclei ligation and reverse crosslinking

Timing: 1.5 days

In the following steps, proximity ligation takes place and chimeric DNA fragments are generated.

13. Centrifuge the nuclei at 900 g for 5 min at 4°C and discard the supernatant.
    a. Add 120 μL of Ligation mix to each sample.

Ligation mix	
Reagent	Amount
10X T4 DNA Ligase Buffer	12 μL
10% Triton X-100	10 μL
10 mg/mL BSA	1.2 μL
ddH_2O nuclease-free	95.8 μL
T4 DNA Ligase (NEB) 2000 CEU/μL	1 μL

Note: Ligase activity can be measured as cohesive-ends (CEU) units or Weiss units (U) depending on the manufacturer. T4 DNA ligase activity from New England Biolabs used in this protocol refers to cohesive-ends unit.

    b. Incubate at 16°C for 16–18 h in a water bath.
14. Centrifuge at 900 g during 5 min at 4°C, discard the supernatant and add 50 μL of 1X Cutsmart (or 1X NEBuffer2).
15. Add 1 μL of 20 mg/mL Proteinase K and incubate for 16–18 h at 65°C to reverse the cross-link.

Pause point: the samples can be stored at −20°C for several weeks.

Hi-C library preparation

Timing: 1.5-2 days

The following steps are performed to immobilize biotinylated DNA fragments for purification and sample preparation/amplification for paired-end sequencing.

16. Purification of Hi-C DNA with magnetic beads.

Note: All the steps are carried out at 20°C–25°C.

    a. Transfer 20 μL of magnetic Streptavidin T1 beads (per single embryo Hi-C sample) to a new DNA low-binding 1.5 mL tube.

Note: Thoroughly mix the magnetic beads to obtain a homogenous suspension.

i. Place the beads suspension on a magnet for 1 min.
ii. Carefully remove the supernatant.

b. Remove the tube from the magnetic stand and add 50 μL of fresh 1X BB + 0.05% Tween 20 (vol/vol).
i. Gently tap the tube to resuspend the beads.
ii. Collect the suspension at the bottom of tube by centrifugation (30 s at 900 g).
iii. Separate the beads on a magnet for 1 min and remove the supernatant.

c. Repeat step 16b once.

d. Remove the tube from the magnetic stand and resuspend the beads in 60 μL of 2X BB.

Note: Prepare fresh 2X BB every time and keep it at 4°C.

e. Transfer 60 μL of the beads suspension to each tube containing the Hi-C material and gently tap the tube to mix.

⚠ CRITICAL: Avoid pipetting! DNA is very dilute and prone to get stuck to pipette tips.

f. Incubate for 1 h at 20°C–25°C on a rotation wheel.

g. Collect the suspension at the bottom of the tube by centrifugation (30 s at 900 g).
i. Separate the beads on a magnet (1 min) and discard the supernatant.
ii. Remove the tube from the magnetic stand and resuspend the beads in 200 μL of 1X BB.

Note: Prepare fresh 1X BB and keep it at 4°C for one week.

h. Collect the suspension at the bottom of the tube by centrifugation (30 s at 900 g).
i. Separate the beads on a magnet (1 min) and discard the supernatant.
ii. Remove the tube from the magnetic stand and resuspend the beads in 200 μL of Elution buffer (EB).

Note: Prepare fresh EB and keep it at 4°C for one week.

17. Enzymatic DNA fragmentation.
a. Collect the suspension at the bottom of tube by centrifugation (30 s at 900 g), separate the beads on a magnet for 1 min and remove the supernatant.
b. Resuspend the beads in 50 μL of AluI digestion mix gently by tapping.

AluI digestion mix

Reagent	Amount
NEBuffer4 10X or Cutsmart 10X	5 μL
ddH_2O nuclease-free	44 μL
AluI (10 U/μL)	1 μL

c. Incubate at 37°C for 1–1.5 h in constant agitation in a microcentrifuge tube thermomixer.

18. Preparation of libraries for sequencing.
a. Place the samples on a magnet, remove the supernatant and resuspend the beads in 200 μL of 1X BB.
b. Place the samples on a magnet, remove the supernatant and resuspend the beads in 200 μL of 1X EB.

c. Place the samples on a magnet, remove supernatant and resuspend the beads in 50 µL of A-tailing mix by tapping gently.

A-tailing mix	
Reagent	Amount
NEBuffer2 10X	5 µL
dATP 10 mM	1 µL
ddH_2O nuclease-free	43 µL
Klenow (3′->5′ exo-) 5 U/µL	1 µL

d. Incubate at 37°C for 30 min in constant agitation in a microcentrifuge tube thermomixer.
e. Separate on a magnet, remove the supernatant and resuspend the beads in 200 µL of 1X BB.
f. Separate on a magnet, remove the supernatant and resuspend the beads in 200 µL of 1X EB.
g. Separate on a magnet, remove the supernatant remove the supernatant and resuspend the beads in 50 µL of adapter ligation mix by pipetting gently.

Adapter ligation mix	
Reagent	Amount
T4 ligase buffer 10X	5 µL
NEBNext adapter (diluted 1/30 from stock)	2.5 µL
ddH_2O nuclease-free	42 µL
T4 DNA ligase (NEB) 2000 U/µL	0.5 µL

Note: Ligation can be left till 16 hours at 20°C–25°C if needed.

⚠ CRITICAL: Sequencing adaptor dilution is essential for reducing adaptor-dimer formation.

h. Incubate at 20°C–25°C for 1 h.
i. Add 2 µL of USER enzyme and incubate at 37°C for 15 min.

Note: USER (Uracil-Specific Excision Reagent) enzyme is used to cleavage the NEBNext hairpin loop adaptor as part of multiplex library preparation for Next Generation Sequencing on the Illumina platform.

j. Separate on a magnet, remove the supernatant and resuspend the beads in 200 µL of 1X BB.
k. Separate on a magnet, remove the supernatant and resuspend the beads in 200 µL of 1X EB.

19. Library amplification and purification.
    a. Separate on magnet the beads and remove the supernatant.
    b. Resuspend the beads in 50 µL of Amplification mix by pipetting gently and transfer the mix to a 0.2 mL PCR tube.

Amplification mix	
Reagent	Amount
NEBNext High-fidelity PCR Master Mix 2X	25 µL
Universal primer	2.5 µL
Indexed primer	2.5 µL
ddH_2O nuclease-free	20 µL

Note: Different indexed primers allow multiplexing of single embryo libraries for Next Generation Sequencing (NGS). Multiplexing of NGS samples makes possible to pool multiple libraries into a single flow cell lane. Each of the indexed primers contains a unique barcode sequence that is used to identify the libraries after sequencing.

c. Amplify the library by PCR:

PCR cycling conditions

Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1
Denaturation	95°C	10 s	15–18 cycles
Annealing	55°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	5 min	1
Hold	4°C	forever	

d. At the end of the PCR reaction, separate the beads on a magnet and transfer the supernatant containing the PCR products to a new DNA low-binding tube. Discard the beads.
e. To purify the library and remove dimers of primers mix the PCR products with 45 μL of AMPure XP beads (0.9x), mix by pipetting and incubate at 20°C–25°C for 5 min.

⚠ CRITICAL: pre-warm AMPure XP beads at 20°C–25°C previously for proper efficiency of purification. For convenience, make an aliquot in advance and keep it at 20°C–25°C.

f. Separate the beads on a magnet for 5 min, discard the supernatant and, while attached to the magnet, wash them with 200 μL of fresh EtOH 70% at 20°C–25°C without mixing.
g. Leave the beads settled on the magnet, remove EtOH and repeat washing with 200 μL of EtOH 70%.

Note: Prepare fresh EtOH 70% and use it at 20°C–25°C.

h. Remove the EtOH and allow the remaining EtOH to evaporate at 20°C–25°C for 5–10 min.

Note: It is important not to over-dry the beads.

i. Add 50 μL of 10 mM Tris-HCl, pH 8.5, mix by gently pipetting and incubate 5 min at 20°C–25°C.
j. Separate the beads on a magnet and transfer the supernatant containing the purified library into a new DNA low-binding tube. Discard the beads.

Note: It is important not to disturb the beads pellet with the pipette tip to avoid transferring of beads together with the supernatant.

k. Add again 45 μL of AMPure XP beads (0.9x) to the supernatant, mix by pipetting and incubate at 20°C–25°C for 5 min.

Note: A second round of AMPure XP beads purification is needed to avoid adaptor- and primer-dimers.

l. Repeat steps 19 f–h.
m. Add 12 μL of 10 mM Tris-HCl, pH 8.5, mix by gently pipetting and incubate 5 min at 20°C–25°C.
n. Separate the beads on a magnet and transfer the supernatant containing the purified library into a new DNA low-binding tube. Discard the beads.

Pause point: Purified libraries can be stored at −20°C for several months.

20. Library quality control.
    a. Use 2 μL of the library for its quantification using a Qbit fluorometer.

Note: A good yield for a single blastocyst Hi-C library would be between 0.1-0.6 ng/μL. Higher concentration of the library is not always indicative of better quality.

    b. Confirm the range of fragment sizes of the libraries by running a High-Sensitivity DNA Bioanalyzer (or equivalent).

Note: The range size of the DNA fragments should be between 200-800 bp (Figure 2).

21. Library sequencing.
    a. Single blastocyst Hi-C libraries should be sequenced at 5–10 million paired-end reads depth with a read length of 50–100 pb.

Note: The reagents used for library preparation are compatible with Illumina sequencing system.

Single embryo Hi-C library genotyping and data processing

Timing: 2–3 days

To optimize the number of libraries generated, we have performed Hi-C in blastocysts from the cross of *Ctcf*$^{fl/-}$; *Zp3-Cre*$^{tg/+}$ females with *Ctcf*$^{fl/-}$ males, which will generate an offspring with 50% of *Ctcf* mutant embryos.[2,3] We also included wild-type embryos (CD-1) as controls.

22. For genotyping on sequence, we considered as mutants those embryos whose libraries contained no reads in the 22 kb of the deleted region (mm10 chr8:105662421-105684451), using other two random regions of the same size (mm10 chr8:122710142-122732172 and chr8:75657421-75679451) as a control. Heterozygous embryos should show half number of reads than wild-type embryos at the deleted region (Figure 3).

Note: Libraries with less than 1 million sequencing reads were excluded to ensure the accuracy of the genotyping.

23. For data analysis, we used TADbit[4] and assess read quality control, read mapping, interaction detection, interaction filtering, and matrix normalization. We followed these steps:
    a. Apply FastQC protocol to discard artifacts and map the remaining reads to the reference mouse genome (mm10).
    b. Discard non-informative contacts including self-circles, dangling-ends, errors, random breaks and duplicates to obtain the valid reads.
    c. Valid pairs from single embryo libraries with the same genotype were merged for the analysis.

Note: We merged around 13 single blastocyst libraries per genotype to perform the subsequent analysis.

    d. As the number of total valid reads can differ among conditions, merged experiments with higher number of reads should be subsampled to have similar number of total reads in all the conditions for a better comparison.

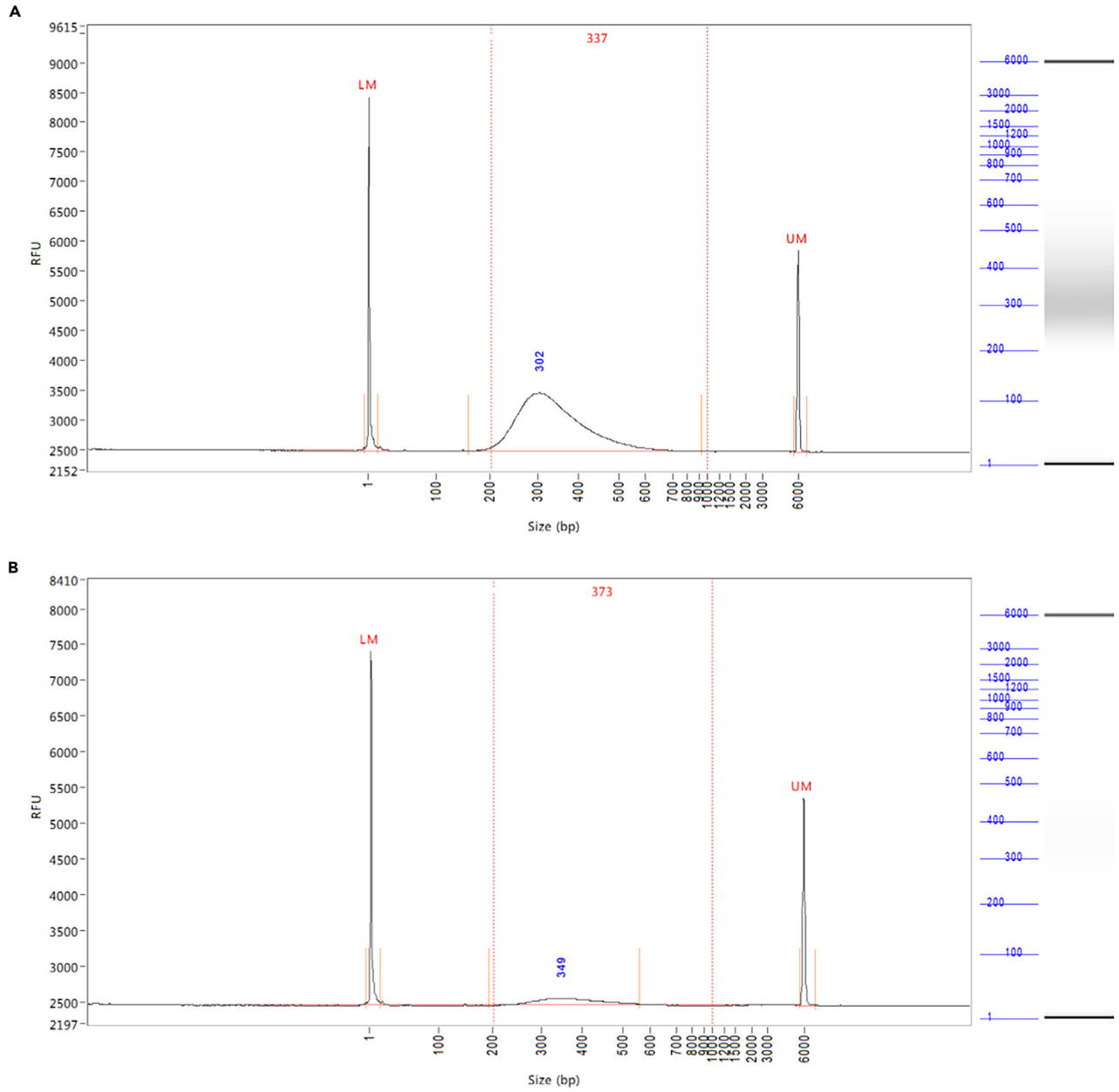

Figure 2. Library quality control

(A and B) Bioanalyzer electropherograms of two Hi-C libraries with proper DNA fragmentation that gave high-quality sequencing data despite presenting different DNA concentration measured by Qbit. LM: Lower Marker; UM: Upper Marker.

Note: There are many other softwares available for processing Hi-C data such as HiC Pro,[7] HiCUP[8] and FAN-C[9] among others.

EXPECTED OUTCOMES

Using the Hi-C protocol described for single mouse blastocysts, more than 2 million valid contacts can be obtained from each high-quality single-embryo library and genotypes can be assigned to each of them with high accuracy. For the comparison among different conditions, merging 12–15 high-quality libraries of each genotype is enough to obtain probability contact matrices of 40-kb resolution

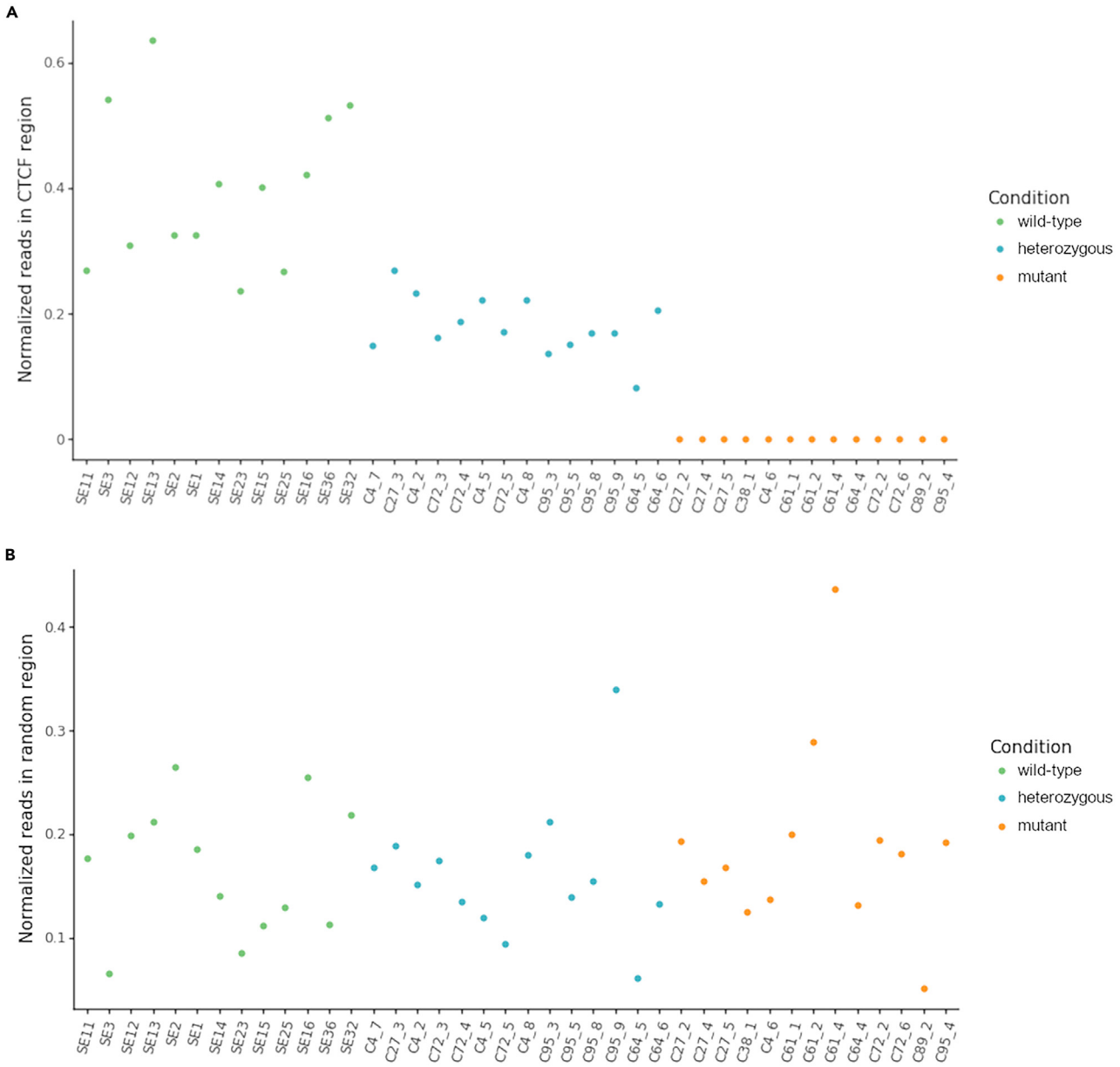

Figure 3. Single embryo Hi-C library genotyping

(A) Normalized number of reads at *Ctcf* genomic region (y-axis) for all single blastocyst Hi-C libraries (x-axis), calculated as the number of reads of the deleted region (mm10 chr8:105662421-105684451) relative to the number of reads of a region of the same size (mm10 chr8:122710142-122732172).

(B) Normalized number of reads at a random genomic region (y-axis) for all single blastocyst Hi-C libraries (x-axis), calculated as the number of reads of the random region (mm10 chr8:75657421-75679451) relative to the number of reads of a region of the same size (mm10 chr8:122710142-122732172). Wild-type embryos are shown in green, heterozygous embryos in blue and *Ctcf* mutant embryos in orange. Adapted with permission from Andreu et al.,[1] Cell Press Publishing Group.

(Figure 4). This resolution is enough to perform comparative analysis of 3D chromatin organization at compartment (10–50 Mb) and TAD (200 kb-1 Mb) levels with confidence (see Andreu et al.[1] for details).

LIMITATIONS

The main limitation of the protocol is the resolution of the contact maps obtained from the single blastocyst Hi-C libraries. The 40-kb resolution achieved is insufficient to

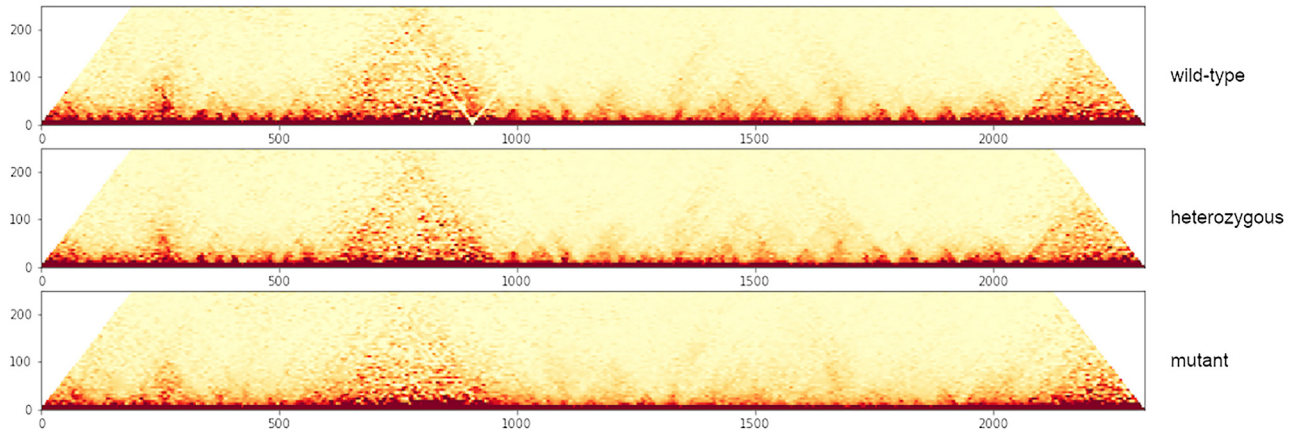

Figure 4. Generation of 3D genome contact maps

Coverage-corrected merged Hi-C matrices of chromosome 17 (mm10 chr17:78000000-90000000) at 40-kb resolution from wild-type (up), heterozygous (middle) and *Ctcf* mutant (down) blastocysts. Visual inspection of merged-by-genotype contact maps reveal a less defined chromatin organization in *Ctcf* mutant blastocysts compared with their littermates (heterozygous) or wild-type embryos. Adapted with permission from Andreu et al.,[1] Cell Press Publishing Group.

perform robust analysis at lower sub-TAD genomic scales, such as loops or enhancer-promoter contacts.

On the other hand, the need to determine the genotype of individual embryos after sequencing and the small amount of starting material when working with blastocysts makes the process inherently inefficient. Only half of the single blastocysts processed resulted in Hi-C libraries with high enough quality for sequencing.

TROUBLESHOOTING

Problem 1

Presence of primer- and/or adapter-dimers (visible in the bioanalyzer electropherogram as 85–90 pb or 127 pb peak respectively, step 20b).

Potential solution

Adapter dimers can affect the depth and quality of sequencing, as they are able to efficiently cluster at the flow cell and become sequenced (thus decreasing the ratio of useful reads). Primer-dimers cannot cluster or be sequenced, but they can bind the flow cell and reduce clustering density so affecting the quality of library sequencing.

Adapter dilution (30-fold) and two rounds of 0.9X AMPure XP beads purification are critical to ensure adapter absence in the library (Figure 5).

Problem 2

Not enough reads for genotyping the library on sequence (<1 million reads, step 22).

Potential solution

Libraries that do not reach the million reads required for accurately genotyping on sequence, but have good quality (30%–40% of valid reads at least) after data processing, can be considered for re-sequencing.

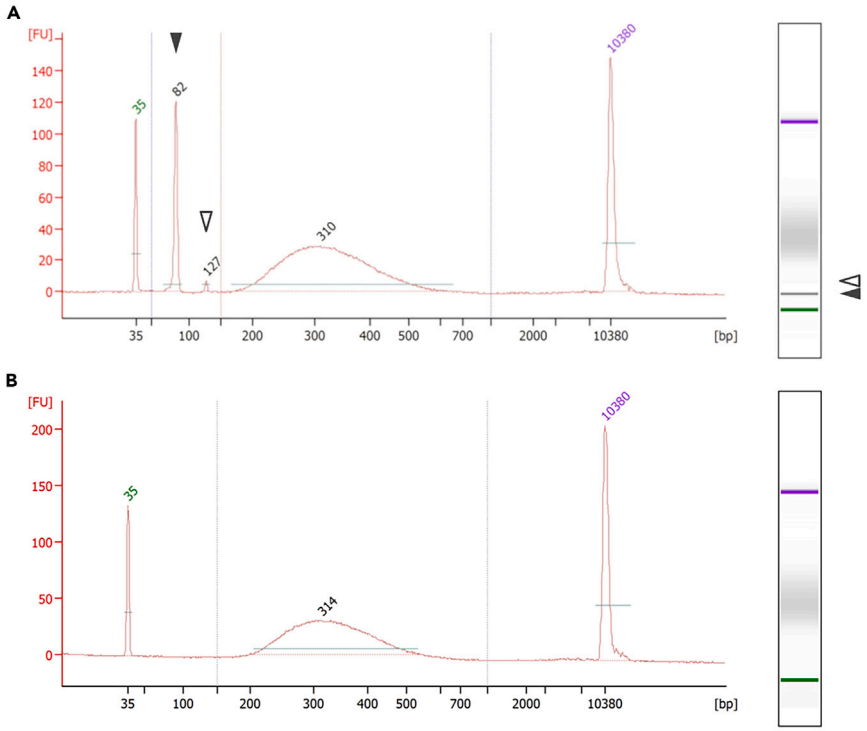

Figure 5. Presence of primer- and/or adapter-dimers in Hi-C libraries

(A and B) Bioanalyzer electropherograms of the same Hi-C library with only one round (A) or with two rounds (B) of AMPure XP beads purification. Solid arrowheads indicate primer-dimers and empty arrowheads indicate adapter-dimers. Peaks at 35 and 10,380 bp are size markers.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Maria Jose Andreu (mandreu@cnio.es).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Original data for figures in the paper is available at https://doi.org/10.1016/j.celrep.2022.111501.
- This paper does not report original code.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.J.A., M.M.; Methodology, M.J.A., D.G.-L.; Writing – Original Draft, D.G.-L., M.J.A.; Writing – Review & Editing, M.J.A., D.G.-L., M.P., A.L., M.M; Funding Acquisition, A.L., M.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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