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Summary

Many population-based methods investigating chromatin dynamics and organization in eukaryotes are based on the Chromosome Conformation Capture (3C) method. Here, we provide an updated version of the quantitative 3C (3C-qPCR) protocol for improved and simplified quantitative analyses of intra-chromosomal contacts.

Key words: Chromosome Conformation Capture, chromatin dynamics and organization, Quantitative PCR, interaction frequency, eukaryotic genome

1. Introduction

Chromosome Conformation Capture (3C) technique *(1)* and derived technologies (4C, 5C, Hi-C) *(2-4)* have allowed researchers to explore the organization and the dynamics of the eukaryotic genomes with unprecedented resolution and accuracy (5-8). The creation of a 3C library is therefore a prerequisite to many 3C-based methods. By freezing all chromatin contacts present at a given time in their physiological nuclear context, and then by averaging these events over a population of several million cells, the 3C-qPCR method *(9)* allows very accurate measurements of the relative interaction frequencies between chromatin segments, in *cis*, on the same chromosome (Figure 1) *(10)*. This parameter is a key to investigate the *in vivo* dynamics of the chromatin because it depends not only on its fundamental biophysical parameters (compaction, rigidity, etc.) *(11)* but also on important locus-specific factors which control local genomic functions (epigenetic modifications, binding of specific factors, etc.) *(12, 13)*. Here we provide an updated 3C-qPCR protocol that simplifies previously published protocols *(9, 14, 15)*.

2. Materials

1. 10% (v/v) FCS/PBS.
2. Homogenization buffer: 2.1M sucrose, 10 mM HEPES (pH7.6), 2 mM EDTA, 15 mM KCl, 10% glycerol, 0.15mM spermine, 0.5 mM DTT, 0.5 mM PMSF, 7 µg/mL aprotinin.

3. Wash buffer: 10 mM Tris-HCl (pH7.4), 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine.
4. Glycerol buffer: 40% (v/v) Glycerol; 50 mM Tris-HCl pH 8.3; 5 mM $MgCl_2$; 0.1 mM EDTA.
5. Nucleus buffer 1: 0.3 M sucrose, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 15 mM Tris-HCl pH7.5, 0.5 mM DTT, 0.1 mM PMSF, 3.6 ng/ml Aprotinin, 5 mM Na-Butyrate.
6. Nucleus buffer 2: nucleus buffer 1 with 0.8% v/v NP40.
7. Nucleus buffer 3: 1.2 M sucrose; 60 mM KCl; 15 mM NaCl; 5 mM $MgCl_2$; 0.1 mM EGTA; 15 mM Tris-HCl pH7.5; 0.5 mM DTT; 0.1 mM PMSF; 3.6 ng/ml Aprotinin; 5 mM Na-Butyrate.
8. 3C buffer: 50 mM Tris-HCl pH 8, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT (see **Note 1**)
9. 37% (v/v) formaldehyde.
10. 1.25 M glycine.
11. 20% (w/v) SDS.
12. Ligation buffer: 40 mM Tris-HCl pH7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP.
13. Triton mix: 10% (v/v) Triton X-100 in ligation buffer.
14. High-concentration restriction enzyme (*e.g.* 40 U/µL HindIII; Fermentas) (see **Note 2**).
15. PK buffer: 10 mM Tris-HCl pH 8, 5 mM EDTA, 0.5% (w/v) SDS.
16. 20 mg/mL proteinase K.
17. 1 mg/mL RNase A.
18. Phenol/chloroform/isoamyl alcohol (25:24:1).

19. 2 M sodium acetate pH 5.6.
20. 100% and 70% ethanol.
21. Secondary digestion restriction enzyme (*e.g.* StyI), supplied with 10X buffer (see **Note 3**).
22. 5 M NaCl.
23. Nuclease-free water.
24. PCR primers, designed according to 3C design (see Methods), including primers loading control primers for loading controls (section 3.8). For human and mouse genomes the following *Gapdh* primers can be used:

    Forward: acagtccatgccatcactgcc

    Reverse: gcctgcttcaccaccttcttg

    Primers used in quantitative PCR are typically 21-23 mers with a Tm in the range 55-65°C with a 2°C maximum difference between primers used in one experiment. They should be designed close (50bp) to the restriction site used for the 3C assays.

25. qPCR mix: SYBR^{R} Green PCR Master Mix as described in *(16)* with modifications described in *(11, 12)* (see **Note 4** for detailed composition).
26. LightCycler 480 II (Roche) or equivalent real-time PCR machine.
27. 30 Weiss U/µL T4 DNA ligase.
28. 100 mM ATP.
29. 20mg/ml glycogen.
30. 10 mM Tris-HCl pH 7.5.

31. Genomic DNA, purified from the same species as for the 3C-qPCR experiments, and quantified by A_{260} measurement.
32. BAC(s), spanning the genomic locus of interest.

3. Methods

3.1 Cell nucleus preparation

1. .- If working with cultured cells, proceed to step 2 below.
   - If working with adherent cell cultures: trypsinize, wash and filter through 40 µm cell strainer to make a single-cell suspension before proceeding with step 2 below (see **Note 5)**.
2. Wash cells in PBS.
3. Resuspend cells in 1.5 ml nucleus buffer 1.
4. Add 0.5 ml nucleus buffer 2, mix slightly and put on ice 2 or 3 minutes.
5. Put 1 ml in 14 ml tubes (x2 tubes) containing 4 ml nucleus buffer 3. Save a 4 µl aliquot to count nuclei in a Thoma cell (count used in step 7 below).
6. Centrifuge 20 minutes at 11300 *g* at 4°C.
7. Remove supernatant and resuspend pellets in glycerol buffer to achieve a final concentration of about 20 million nuclei in 100 µl.
8. Transfer in a 1.5 mL tube. Freeze into liquid nitrogen and store at -80°C.

3.2 Formaldehyde crosslinking

1. Resuspend $5x10^6$ nuclei in 700 µl 3C buffer and incubate for 5 min at room temperature.

2. Add 19.7 µL 37% formaldehyde, mix by inverting the tube and incubate for 10 min at room temperature.
3. Add 80 µL 1.25 M glycine, mix by inverting the tube and incubate for 2 min at room temperature then place on ice for 5 min.
4. Centrifuge for 3 min at 2300 *g*, room temperature, remove supernatant and carefully resuspend nuclei in 1 mL 3C buffer (*see* **Note 6**).
5. Centrifuge for 3 min at 2300 *g,* room temperature and remove supernatant.

3.3 Restriction digestion

1. Resuspend nuclei in 100 µL 3C buffer and transfer to a safe-lock microtube.
2. Add 1 µL 20% SDS and incubate for 1 h at 37°C, shaking at 350 rpm on a thermomixer.
3. Add 16.8 µL Triton mix and incubate for 1 h at 37°C, shaking at 350 rpm on a thermomixer.
4. Take a 10 µL aliquot of the sample as the “Undigested control” (do not disturb the mixture) and store at -20°C until processing at section **3.4**.
5. Add 450 U of the selected restriction enzyme (*eg*. HindIII) to the remaining sample and incubate overnight at 37°C shaking at 350 rpm on a thermomixer (*see* **Note 2**).
6. Take a 10 µL aliquot of the sample as the “Digested control” (do not disturb the mixture) and proceed with this to section **3.4**. The remaining sample is processed in section **3.5**.

3.4 Determination of digestion efficiency

Digestion efficiencies have a significant impact on the assays and should be carefully assessed for each restriction site involved in the analysis. Care should be taken to ensure that digestion efficiencies are in the same range for the sites of interest (see **Note 2**).

1. Add 500 µL PK buffer and 1 µL 20 mg/mL proteinase K to the “Undigested” and “Digested” control samples, and incubate for 30 min at 65°C.
2. Cool samples to 37°C, add 1 µL 1 mg/mL RNase A and incubate for 2 h at 37°C.
3. Add 500 µL phenol/chloroform/isoamyl alcohol, mix vigorously and centrifuge for 5 min at 16,100 *g*, room temperature.
4. Transfer upper aqueous layer to a new microtube. Add 50 µL 2 M sodium acetate and 1.5 mL 100% ethanol and keep at -80°C for 45 min.
5. Centrifuge for 20 min at 16,100 *g*, 4°C, carefully remove supernatant and wash pellet in 500 µL 70% ethanol.
6. Centrifuge for 4 min at 16,100 *g*, room temperature, remove supernatant and air-dry the pellet.
7. Dissolve DNA in 500 µL 1X secondary digestion restriction enzyme buffer. Add 50 U secondary digestion restriction enzyme and incubate for 2 h at 37°C (*see* **Note 7**).
8. Add 500 µL phenol/chloroform/isoamyl alcohol, mix vigorously and centrifuge for 5 min at 16,100 *g*, room temperature.
9. Transfer upper aqueous layer to a new microtube. Add 25 µL 5 M NaCl and 1 mL 100% ethanol and keep overnight at -20°C.
10. Centrifuge for 20 min at 16,100 *g*, 4°C, remove supernatant and wash pellet in 200 µL 70% ethanol.

11. Centrifuge for 4 min at 16,100 *g*, room temperature, remove supernatant, and air-dry then dissolve pellet in 60 µL nuclease-free water.
12. Perform real-time PCR quantification (see **Note 4** and section 3.10 below) on undigested (U) and digested (D) control samples, using primer sets that amplify across each restriction site of interest (R). To correct for any difference in the amounts of templates used in the PCR, also use primer sets to amplify control regions (C) that do not contain the restriction sites of interest. Digestion efficiency is calculated according to the following formula (*see* **Note 8**):

    % restriction = 100 – 100 / 2^ ($(Ct_R - Ct_C)_D - (Ct_R - Ct_C)_U$)

3.5 Ligation

1. Add 12 µL 20% SDS to the digested sample and incubate for 30 min at 37°C, shaking on a thermomixer at 350 rpm.
2. Carefully transfer digested nuclei to a 12 mL tube (*see* **Note 9**). Add 3.28 mL ligation buffer and 390 µL Triton mix, and incubate for 2 h at 37°C, shaking in a thermomixer at 450 rpm.
3. Centrifuge for 1 min at 8800 *g*, 4°C and remove 3.27 mL supernatant to leave 500 µL in the tube. Add 6.5 µL 30 U/µL T4 DNA ligase and 3 µL 100 mM ATP and incubate overnight at 16°C, shaking on a thermomixer at 350 rpm.

3.6 DNA purification

1. Add 2 mL PK buffer, 1.5 mL nuclease-free water and 5 µL 20 mg/mL proteinase K, and incubate for 1 h at 50°C.
2. Incubate for 4 h at 65°C to de-crosslink the sample.

3. Add 4 mL phenol/chloroform/isoamyl alcohol, mix vigorously and centrifuge for 15 min at 3900 *g*, room temperature (*see* **Note 10**).
4. Transfer upper aqueous layer to a new 12 mL tube and add 200 µL 5 M NaCl, 1 µL glycogen and 8 mL 100% ethanol. Mix and store overnight at -20°C.
5. Centrifuge for 45 min at 15,700 *g*, 4°C, remove supernatant and wash pellet in 2 mL 70% ethanol.
6. Centrifuge for 15 min at 15,700 *g*, 4°C, remove supernatant, air-dry pellet and dissolve DNA in 50 µL nuclease-free water (see **Note 11**).

3.7 Complementary digestion

1. Add 250 µL 2X secondary digestion restriction enzyme buffer (*see* **Note 3**) and 185 µL nuclease-free water, and transfer sample to a 1.5 mL microtube.
2. Add 5 µL 1 mg/mL RNase A and 100 U secondary digestion restriction enzyme, and incubate for 2 h 30 min at 37°C.
3. Add 500 µL phenol/chloroform/isoamyl alcohol, mix vigorousy and centrifuge for 5 min at 3900 *g*, room temperature.
4. Transfer upper aqueous phase to a new 1.5 mL tube and add 25 µL 5 M NaCl, 1 µL glycogen and 1 mL 100% ethanol, and keep overnight at -20°C.
5. Centrifuge for 20 min at 15,700 *g*, 4°C, remove supernatant and wash pellet in 200 µL 70% ethanol.
6. Centrifuge for 4 min at 15,700 *g*, room temperature, air-dry pellet and dissolve DNA in 150 µL 10 mM Tris-HCl pH 7.5 (*see* **Note 11**).

3.8 Performing loading adjustments

1. Determine the DNA concentration of the 3C sample by qPCR (*see* **Note 4** and **Note 12**), relative to a reference genomic DNA of known concentration. Use a dilution series of 3C material and genomic DNA, and use “internal” primer sets that do not amplify across sites recognized by any of the restriction enzymes used. Set up 10 µL reaction volumes in qPCR plates:

   1 µL DNA (3C or genomic, or nuclease-free water in negative control well);

   7 µL nuclease-free water;

   1 µL 5 µM each primer;

   1 µL qPCR mix (see **Note 4**).

2. Perform the following programme in a qPCR thermal cycler (*see* **Note 12**):

   95°C, 3 min;

   45 x [95°C, 1 s; 60°C, 5 s; 72°C, 15 s]

   Denaturation curve: 45°C, 30 s; increase to 95°C at 0.2°C/s.

3. Use the amplification curve from the known genomic DNA concentrations to estimate the concentration of the 3C DNA samples. Dilute the 3C samples to 25 ng/µL.

4. Repeat steps 1-2 on the new 3C stocks to precisely determine their concentration. These values will be used as the “loading controls” for final quantification (see **3.12**).

3.9 Assessment of sample purity (optional)

1. Take a two-fold and a four-fold diluted aliquot of the 3C sample (**section 3.7**, step 6) and add genomic DNA to each aliquot to make a final total DNA concentration of 25 ng/µL.
2. Perform qPCR for any 3C primer pair (see **Note 4** and section 3.10 below) and verify that the real-time PCR quantifications are reduced according to the dilution factors. If this is not the case, then sample purity is not adequate and the DNA should either be re-purified or the sample discarded.

3.10 Real-time PCR quantifications of ligation products

3. Perform qPCR reactions to obtain the Ct for each assessed ligation product on 25 ng 3C material. Set up 10 µL reaction volumes in qPCR plates:

    1 µL DNA (3C, or nuclease-free water in negative control well);

    7 µL nuclease-free water;

    1 µL 5 µM each primer;

    1 µL qPCR mix (*see* **Note 4**).

    1. Perform the following programme in a qPCR thermal cycler (*see* **Note 12**):

        95°C, 10 min;

        45 x: [95°C, 10 s; 69°C, 8 s; 72°C, 14 s].

3.11 PCR control template used for primer efficiency control

A control template containing all ligation products in equal amounts is used to optimise real-time quantitative PCR (qPCR) reactions and to establish the minimal amount of ligation product that can still be quantified in a reliable manner. For this qPCR control template, we recommend the use of a single BAC clone covering the

genome segment under study. Alternatively, a set of minimally overlapping BAC clones mixed in equimolar amounts can be used. This BAC is then cut with the 3C restriction enzyme of choice (*eg*. HindIII) and religated by T4 DNA ligase. A secondary restriction enzyme (*eg.* StyI) can be used to linearize DNA circles which may otherwise affect primer hybridisation efficiency *(17)* (see **Note 3**). It is then necessary to make serial dilutions of this reaction to obtain standard curves which cover the same range of ligation product concentrations as those that will be obtained in the 3C samples. To mimic 3C sample conditions, the final DNA concentration in these dilutions is adjusted to the amount of DNA used in the 3C samples. Thus, these dilutions are performed in a 25 ng/µL DNA solution made of genomic DNA digested with the second restriction enzyme (*eg.* StyI in the present protocol). Using serial dilutions of this control template, a standard curve with specific parameters (slope and intercept) is thus obtained for each of the 3C qPCR primer pairs used. These parameters will be used to correct for potential differences in primer efficiencies.

3.12 3C-qPCR data normalization – primer efficiency and loading controls

To obtain quantification values that are corrected for potential differences in primer efficiencies, the Ct obtained for each ligation product is first normalized using the parameters of the corresponding standard curve (the slope "*a*" and the intercept "*b*" obtained in section **3.11**). These values are calculated using the following formula: Value = $10^{(Ct-b)/a}$. For each sample, these values are then normalized to the corresponding "loading control" obtained in section **3.8**.

3.13 3C-qPCR data normalization to noise band

The data should then be normalized to compensate for experimental variations and allow comparison between different 3C-assays. For each biological sample, a Basal Interaction Level (B.I.L.) is calculated and 3C-qPCR data are normalized to this B.I.L.. We first calculate the mean interaction frequency (M) and the mean Standard Deviation (SD) of all the experimental points. Experimental points are selected if their interaction frequency (fx) is both superior to (M – SD) and inferior to (M + SD). The mean fx of the selected experimental points is corresponding to the B.I.L. to which all fx values of the experiment are normalized *(14, 15)*.

4. Notes

1. This buffer is intended to be compatible with the selected restriction enzyme and should thus be adapted accordingly.
2. Digestion efficiency in 3C experimental conditions varies largely depending on the selected restriction enzyme. Some enzymes, like HindIII or EcoRI, have high digestion efficiencies (at least 70 to 80% is recommended for all sites investigated in a given experiment) while others, like BamHI, require sequential addition of the 450U to reach the same efficiency (add 150U for 2h, then 150U for 2h, and finally 150U overnight). Some enzyme, like SacI, does not digest at all DNA in 3C reactions. Enzymes that generate cohesive ends are recommended, as blunted ends do not allow efficient ligation.

3. This enzyme is required to fragment the DNA which help to improve DNA accessibility and thus PCR amplification efficiencies. Please insure that this enzyme does not digest within the tested amplicons.
4. Quantitative PCR mix composition *(16)*: 0.24% W1 (polyoxyethylene ether W1); 500 µg/ml BSA; 300 µM dNTP; 50 mM KCl; 30 mM $MgCl_2$; 1/3000 SYB^R Green (10000X in DMSO, LONZA, ref. 50513); 16.24% Glycerol; 400 mM 2-Amino-2-methyl-1,3-propanediol buffer to pH 8.3 using HCl; 0.4 U/µl platinium Taq DNA polymerase (Invitrogen 10966-034). Use a fresh glycerol stock, as glycerol tends to produce oxidization products that inhibit PCR reactions. W1 can be replaced by a combination of two detergents: 0.09% Brij 56 (Sigma P5759) and 0.15% Brij 58P (Sigma P5884). For better quantification efficiencies, the usual 300 µM dNTP could be replaced by 1500 µM CleanAmp 3'THF dNTP (Tebu Bio). We do not recommend the use of CleanAmp dNTP 3'TBE, as they provide similar quantification efficiencies as classical dNTP
5. Nuclei preparation from tissues requires specific adaptations. For example, for mouse liver: homogenize the tissue in homogenization buffer (see section 2 for buffer composition) using a Potter. Deposit the homogenate on a cushion of the same buffer and centrifugate at 100,000*g* for 40 min. Wash the pellet with wash buffer and resuspend in glycerol buffer. 9. Freeze into liquid nitrogen and store at -80°C. For muscle, the same protocol can be used except that the tissue needs to be first disrupted using an ultra-turrax before homogenization *(18,19)*.
6. Rinse the walls of the tube that have been in contact with the reaction mixture to recover all nuclei.

7. Check that the corresponding secondary digestion restriction site is absent from the PCR amplicons used to assess digestion efficiencies.
8. The efficiency of the restriction enzyme digestion should be above 60-70%, but ideally >80% is digested. Samples with lower digestion efficiencies should be discarded (also see **Note 2**).
9. Do not rinse the wall of the tube as this usually increase significantly the amount of undigested material.
10. If the aqueous phase if very turbid after the first extraction, repeat the phenol-chloroform extraction a second time.
11. If some precipitates do not resuspend, dissolve DNA by gently shaking tubes at 37°C for up to 30 min. The 3C template may be kept at -20°C for several months.
12. A_{260} measurements fail to provide an accurate estimate of DNA concentration in 3C samples, probably because of their limited purity. If qPCR reactions are performed in a different thermocycler (than the LightCycler, Roche) the PCR parameters may need to be optimised.

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References

1. Dekker J, Rippe K, Dekker M et al. (2002) Capturing chromosome conformation. *Science.* 295: 1306-1311.

2. Dostie J, Richmond TA, Arnaoult RA et al. (2006) Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. *Genome Res.* 16: 1299-1309. .

3. Simonis M, Klous P, Splinter E et al. (2006) Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat Genet.* 38: 1348-1354.

4. Zhao Z, Tavoosidana G, Sjolinder M et al. (2006) Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. *Nat Genet.* 38: 1341-1347.

5. Dixon JR, Selvaraj S, Yue F et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485: 376-380. .

6. Duan Z, Andronescu M, Schutz K et al. (2010) A three-dimensional model of the yeast genome. *Nature* 465: 363-367.

7. Lieberman-Aiden E, van Berkum NL, Williams L et al. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science.* 326: 289-293.

8. Sexton T, Yaffe E, Kenigsberg E et al. (2012) Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* 148: 458-472.

9. Hagège H, Klous P, Braem C et al. (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat Protoc* 2: 1722-1733.

10. Ea V, Baudement MO, Lesne A et al. (2015) Contribution of Topological Domains and Loop Formation to 3D Chromatin Organization. *Genes (Basel).* 6: 734-750.

11. Court F, Miro J, Braem C et al. (2011a) Modulated contact frequencies at gene-rich loci support a statistical helix model for mammalian chromatin organization. *Genome Biol* 12: R42. .

12. Court F, et al. (2011b) Long-range chromatin interactions at the mouse Igf2/H19 locus reveal a novel paternally expressed long non-coding RNA. *Nucleic Acids Res* 39: 5893-5906.

13. Ea V, Sexton T, Gostan T et al. (2015) Distinct polymer physics principles govern chromatin dynamics in mouse and Drosophila topological domains. *BMC Genomics.* 16: 607. doi: 610.1186/s12864-12015-11786-12868.

14. Braem C, Recolin B, Rancourt RC et al. (2008) Genomic matrix attachment region and chromosome conformation capture quantitative real time PCR assays identify novel putative regulatory elements at the imprinted Dlk1/Gtl2 locus. *J Biol Chem* 283: 18612-18620.

15. Ea V, Court F, Forné T (2017) Quantitative Analysis of Intra-chromosomal Contacts: The 3C-qPCR Method. *Methods Mol Biol* 1589: 75-88.

16. Lutfalla G, Uzé G (2006) Performing quantitative reverse-transcribed polymerase chain reaction experiments. (Translated from eng) *Methods Enzymol* 410: 386-400 (in eng).

17. Weber M, Hagège H, Lutfalla G et al. (2003) A real-time polymerase chain reaction assay for quantification of allele ratios and correction of amplification bias. *Anal Biochem.* 320: 252-258.

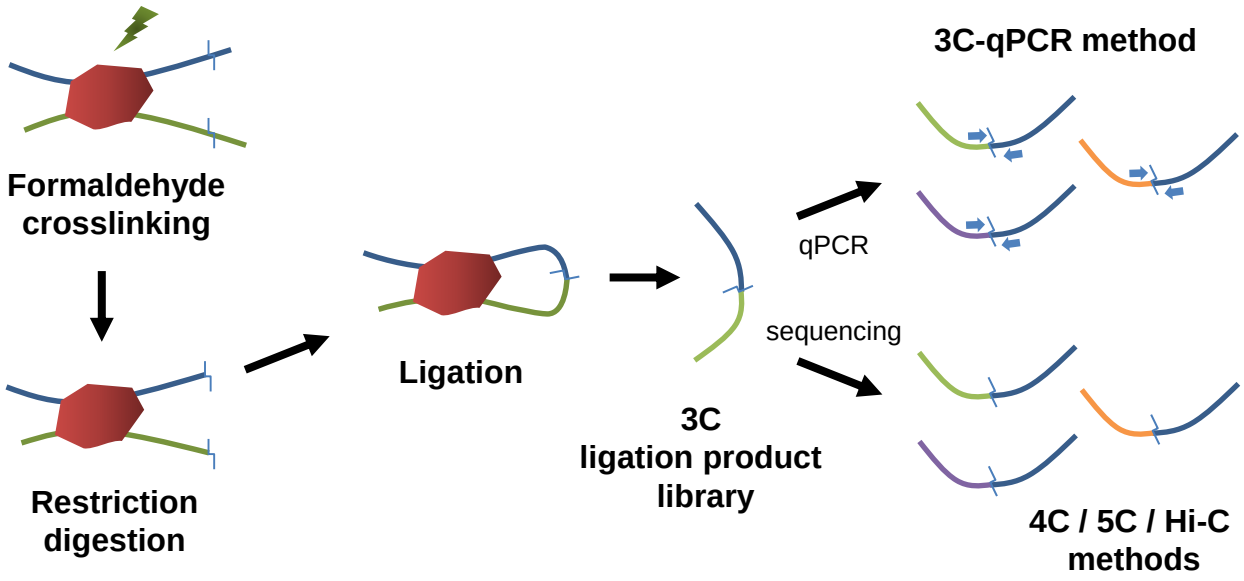
18. Milligan L, Antoine E, Bisbal C et al. (2000) H19 gene expression is up-regulated exclusively by stabilization of the RNA during muscle cell differentiation. *Oncogene.* 19: 5810-5816.

19. Milligan L, Forné T, Antoine E et al. (2002) Turnover of primary transcripts is a major step in the regulation of mouse H19 gene expression. *EMBO Rep.* 3: 774-779.

Figure caption

Figure 1: Principle of the Chromosome Conformation Capture (3C) assay. The principle of the 3C technique relies on three essential steps: formaldehyde crosslinking, restriction digestion and ligation, providing a library composed of 3C ligation products. The relative interaction frequency of each ligated DNA fragments, that reflect their 3D physical proximity, can be accurately determined by quantitative PCR (3C-qPCR method). 3C libraries can also be combined with high-throughput sequencing approaches and used for other 3C based methods, like the 4C, 5C or Hi-C assays (see text for references).

FIGURE 1


Formaldehyde crosslinking
Restriction digestion
Ligation
3C ligation product library
qPCR
sequencing
3C-qPCR method
4C / 5C / Hi-C methods