Chapter 14

Technical Review: A Hitchhiker's Guide to Chromosome Conformation Capture

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Abstract

The introduction of chromosome conformation capture (3C) technologies boosted the field of 3D-genome research and significantly enhanced the available toolset to study chromosomal architecture. 3C technologies not only offer increased resolution compared to the previously dominant cytological approaches but also allow the simultaneous study of genome-wide 3D chromatin contacts, thereby enabling a candidate-free perspective on 3D-genome architecture. Since its introduction in 2002, 3C technologies evolved rapidly and now constitute a collection of tools, each with their strengths and pitfalls with respect to specific research questions. This chapter aims at guiding 3C novices through the labyrinth of potential applications of the various family members, hopefully providing a valuable basis for choosing the appropriate strategy for different research questions.

Key words Chromosome Conformation Capture, Chromosome Conformation Carbon Copy, Hi-C, ChIA-PET, 3D genome organization, HiChIP, Capture Hi-C, Targeted Chromatin Capture

1 Introduction

In recent years, the study of 3D-genome architecture became one of the leading fields in genome research, providing novel insights into 3D genome organization and its effect on the cell's functions. As the field is rather young, our knowledge on the influence of the 3D-genome on genome functions, such as transcription, replication, and epigenetic inheritance of cellular states is only emerging, but an increasing number of studies are focusing on 3D-genome architecture and its functional consequence. Alongside with microscopy approaches, Chromosome Conformation Capture (3C) technologies are the most important drivers of this research field. To date, the 3C collection comprises six main members, varying in their suitability for specific research questions. 3C technologies are based on a common experimental protocol, which involves formaldehyde cross-linking of native chromatin to capture 3D chromosomal interactions, followed by either enzymatic or

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physical chromatin fragmentation and proximity ligation. This leads to the production of hybrid DNA molecules, termed as 3C templates, which are indicative for specific 3D-chromatin contacts. 3C templates typically include two DNA fragments, which were in contact in the 3D-space. The relative abundance of a given 3C template is then used to determine an averaged contact frequency across the cell population. The fragment size ultimately defines the resolution with which 3D-contacts can be analyzed. The downstream sample preparation and, most importantly, the readout of each assay distinguish the various members, which are discussed below. Hence, depending on the desired information, a certain 3C method is preferred for a given research question.

A number of 3C technologies require sophisticated bioinformatics approaches for data analysis. A comprehensive list and description of tools can be found at https://omictools.com/3c-4c-5c-hi-c-chia-pet-category.

1.1 Chromosome Conformation Capture (3C)

- *Type*: One-to-one.
- *Application*: Analyzing specific pairwise chromosomal 3D-contacts.
- *Protocols*: [1–5] and *see* Chapter 15 [6].

3C [7], the founding member of the 3C family, is employed to analyze the contact frequency of a pair of chromosomal regions (e.g., an enhancer and a promoter region) and, thus, represents a candidate approach to study specific chromatin interactions (*see* Fig. 1). 3C templates representing the regions of interest are PCR amplified using primers specific to each of the candidate regions (*see* Fig. 2). The primers are typically designed in parallel to each other (i.e., facing in the same direction) to ensure that only valid 3C templates, which underwent digestion and religation, can be amplified. The contact intensity is determined either semiquantitatively by the intensity of a band on an agarose gel or quantitatively by qPCR [5]. The obtained values are then typically compared to pairs of neighboring genomic regions, which are thought to exhibit lower contact frequencies (e.g., a region next to the enhancer element or adjacent to the promoter).

The correct application of 3C may be challenging. As the primers used to amplify the 3C template of interest and the primers used to amplify control 3C templates are not identical, utmost caution is essential to ensure appropriate PCR conditions and comparable primer efficiency [4]. Especially ensuring equal primer efficiency can be very tedious, as 3C PCR primers can neither be assessed on 3C DNA (as the amount of template is unknown), nor on genomic DNA (as 3C primers are designed in parallel and, thus, cannot amplify genomic sequences). Hence, known amounts of 3C-like templates have to be generated. This can be accomplished

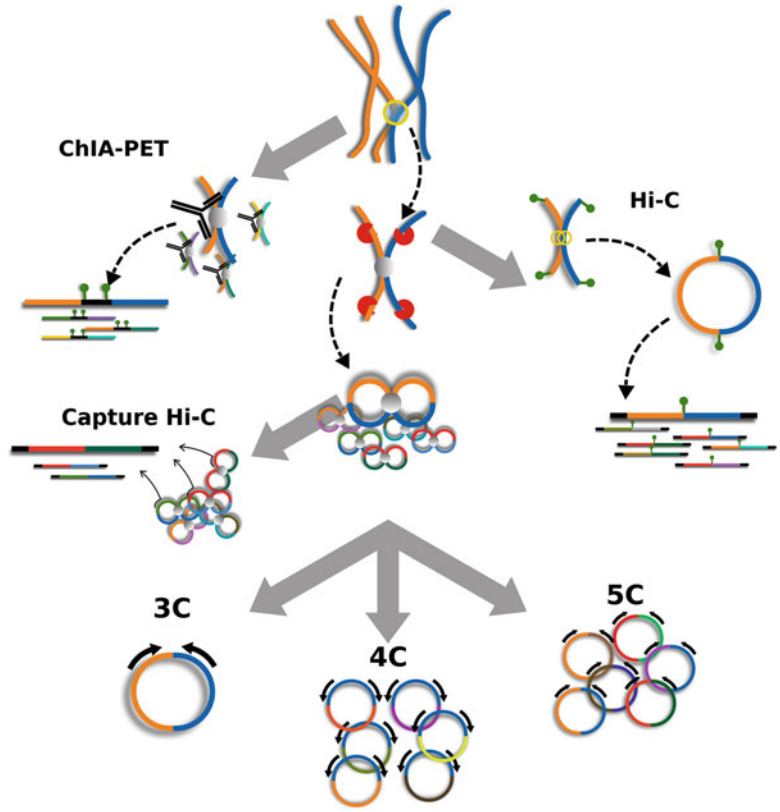

Fig. 1 Overview of the set of 3C technologies

by cloning of the expected 3C templates or by digestion and random religation of bacterial artificial chromosomes (BACs), covering the chromosomal region of interest. Furthermore, a number of control 3C templates originating from 3D-contacts adjacent to the candidate pair need to be included, leading to a substantial number of primer pairs, whose efficiency must be faithfully assessed using the above described strategies. However, once the experimental setup is established, various experimental conditions, such as comparisons between tissues or wild type and mutant genetic backgrounds can be investigated simultaneously without the need of sophisticated statistical analysis of the retrieved data.

In summary, performing standard 3C can be rather time consuming, thus, it is best applied to strong candidate regions rather than to screen for chromosomal contacts across large genomic domains. Since the downstream analysis of the retrieved data does not require profound knowledge on bio-statistics, 3C can be performed by any experienced molecular biologist without the need to engage bioinformatics specialists.

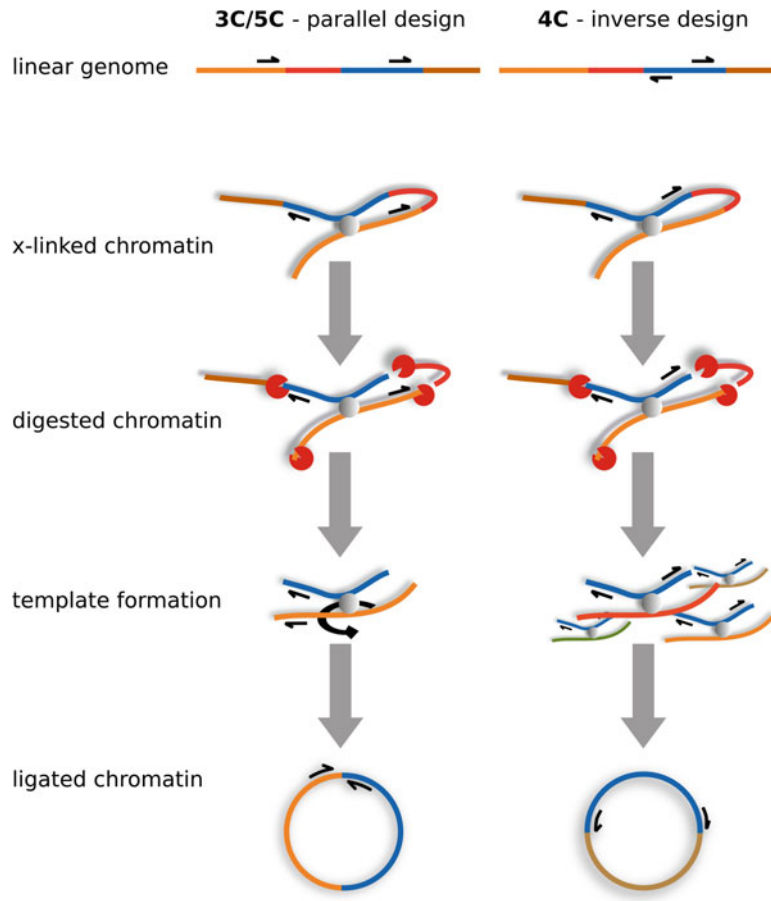

Fig. 2 Primer design. 3C, 4C, and 5C template amplification requires specific primer design, allowing specific amplification of informative 3C templates and preventing amplification of genomic DNA templates, which occurs in most 3C preparations due to incomplete chromatin digestion. Note in step "template formation": The cross-linking site acts like a hinge, around which the two fragments can turn and, thus, all fragment ends can ligate to each other

In plants, 3C has proven its power by important discoveries. In its first application in plants, 3C has been employed to describe the involvement of 3D contacts in the regulation of paramutagenic loci in maize [8]. Later, 3C allowed the identification of a vernalization-dependent gene loop linking 5′ and 3′ flanking regions of the flowering regulator *FLC* [9]. Additionally, 3C has allowed the characterization of chromatin loop involved in the regulation of the noncoding RNA *APOLO* expression [10].

1.2 Chromosome Conformation Carbon Copy (5C)

- *Type*: Many-to-many.
- *Application*: Comprehensive assessment of all 3D-contacts within a chromosomal region of interest.
- *Protocols*: [11–13].

5C [14] is the method of choice to comprehensively characterize all 3D-contacts of a chromosomal region of limited size up to a few megabases (*see* Fig. 1). Chromosomal regions, such as gene clusters, can engage in complex 3D-looping structures, connecting several regulatory and effector regions in 3D-space. Hence, the detailed analysis of all possible 3D-contacts in such a region can be of special interest. Methodologically, 5C is closely related to 3C. However, instead of using single primer pairs to amplify specific 3C templates characteristic for specific 3D-interactions, all 3C templates originating from the region of interest are amplified and later quantified using either microarray or next-generation sequencing technology. To amplify the entire interactome of the region of interest, primers specifically annealing to each restriction fragment are designed. To avoid amplification of circularized self-ligated fragments, primers are designed in parallel (i.e., pointing in the same direction) in order to only amplify head-to-head 3C ligation products (*see* Fig. 2). Furthermore, the specific primers carry tails common to all forward or reverse primers. This design subsequently allows for ligation-mediated amplification (LMA) of 3C templates within the region of interest. Similar to 3C, primer annealing efficiency has to be tightly controlled, as it is likely to vary among the pool of primers employed in the experiment. Control PCR amplification is achieved using digested and randomly religated BAC clones covering the region of interest.

Importantly, the complexity of the obtained 5C data increases exponentially with the number of fragments located in the region of interest. Analyzing a region of 100 fragments will generate 10,000 contact frequencies, whereas a region of 1000 fragments will yield 1,000,000 possible individual contacts. Hence, to obtain a satisfactory sequencing coverage, the number of fragments occurring in the region of interest has to be taken into consideration. Various bioinformatics tools were engineered to analyze 5C contact data (e.g., [13, 15]). In principle, most Hi-C (*see* below) data analysis tools can also be employed to process 5C data.

5C can generate 3D-contact maps of specific regions at unparalleled resolution. However, the design and generation of a large number of primers can be cost-intensive. Therefore, especially in case of relatively small genomes such as those of yeast, *Drosophila melanogaster*, or *Arabidopsis thaliana*, it may be advisable to perform Hi-C technology (*see* below) at high resolution, which may yield comparable resolution and cost for the whole genome. Furthermore, 5C technology is today in competition with a novel variant of Hi-C, termed Capture Hi-C (*see* below), which may produce comparable output at significantly lower cost.

Although widely employed in metazoen models, to our knowledge there has been no study using 5C published to date in the plant field. However, 5C may be beneficial to unravel complex folding principles of certain genomic loci. To date, little is known

about enhancer–promoter interactions. 5C or Capture Hi-C (*see* below) may significantly advance how local chromatin folding contributes to transcriptional regulation in plants.

1.3 Circular Chromosome Conformation Capture/ Chromosome Conformation Capture on Chip (4C)

- *Type*: One-to-all.
- *Application*: Assessment of genome-wide chromosomal contacts of a candidate region.
- *Protocols*: [16–19].

4C [20, 21] is used to screen the genome for regions that specifically contact a candidate region in 3D-space (*see* Fig. 1). The search for interacting regulatory regions, such as enhancers, for a given chromosomal region of interest (e.g., a promoter) is one of the most prominent applications of 4C.

4C technology involves specific amplification of all 3C templates associated with a given region of interest (*see* Fig. 2). Thereby, an inverse PCR scheme is designed using primers specific for a candidate fragment, termed "bait" or "viewpoint." The primers anneal to the viewpoint's fragment ends pointing "outwards." This allows for amplification of all "prey" DNA fragments that interact with the viewpoint in 3D. As many 3C templates that were generated by a 6-cutter restriction enzyme may be comprised of prey fragments too large for efficient amplification, the 3C templates are subject to a second fragmentation and religation step, typically using a 4-cutter restriction enzyme. The composition of the final PCR products are then quantified using either microarrays or, more commonly, high-throughput sequencing. The obtained data are used to create a 4C-profile, which is typically represented by peaks corresponding to the contact frequency along the genome. The resolution of genome-wide 3D-contacts depends on sequencing coverage, which in turn is determined by the number of fragments generated during the primary 3C template generation (which is determined by the size of the genome and the frequency of restriction sites within). Hence, assuming a genome size of 1Gb, using state-of-the-art sequencing technology (e.g., one lane of Illumina sequencing) usually provides single fragment resolution, which is sufficient to analyze most biologically relevant chromosomal interactions.

A variant of 4C, termed enhanced 4C (e4C) [18], reduces library complexity by specific enrichment for a subset of chromatin associations and, thus, either significantly increases resolution at constant sequencing depth, or allows employing inexpensive microarray technology to retrieve the 4C experimental output.

The downstream assessment of 4C sequencing reads requires sequencing data processing and statistics and, thus, some skills in computational data analysis. Today, various computational tools are available (e.g., [22, 23]), which can also be used by scientists with

relatively little experience in computational biology. To extract biologically significant 3D-contacts, the data sets have to be normalized for elevated contact frequencies that rely on linear proximity on the DNA. Neighboring genomic regions generally exhibit high 3D-contact frequencies, from which little biological significance can be inferred. However, they may mask biologically significant 3D-contacts, hence the need to normalize for linear distance-dependent 3D-contact frequencies. This normalization may be challenging especially in close proximity to the viewpoint, making it difficult to identify prey regions in close linear proximity to the viewpoint. Thus, 4C may not be suitable to analyze short-range interactions of less than approximately 50 kb.

Similar to 3C, 4C can easily be applied to study 3D-genome architecture in different experimental conditions. For many research questions, 4C data from various conditions can be compared using robust statistical software, designed for differential analysis of expression data (e.g., [24]). 4C technology is robust and can also be applied simultaneously to various viewpoints, which can be investigated in the same 4C sample using the primer sequences as bar codes. Although 4C can provide a 3D-contact profile in a rather short time and with limited financial requirements, it is not optimally suited to analyze 3D-contacts over short linear distances.

Despite the wide range of potential applications of 4C in plants, to our knowledge, only one report employing 4C has been published to date [17]. In a rather atypical application, general features of chromosomal architecture have been described based on 4C data. This study shows that 4C cannot only be beneficial to detect specific interactors of a given viewpoint, but, moreover, can also be deployed to reveal basic principles of chromosomal organization, such as the relationship of heterochromatin and euchromatin in 3D.

1.4 Genome-Wide 3C (Hi-C)

- *Type*: All-to-all.
- *Application*: Characterization of global chromatin contacts, assembly of genomes.
- *Protocols*: [25–29].

Hi-C [30] is designed to generate 3D-contact maps of entire genomes and catches all possible genome-wide chromatin interactions (*see* Fig. 1). Using Hi-C, basic organizational principles of genome organization were revealed [30, 31], significantly advancing the chromosomal architecture field. The identification of genome-wide contact frequencies can also assist in de novo genome assembly [32–35], using the fact that contigs in linear genomic neighborhood theoretically exhibit the highest contact frequencies among each other. Hence, by incorporation of the acquired contact

frequencies, contigs of previously unknown genomic position can be put into linear relation.

In the Hi-C experimental protocol, successfully digested and religated fragments are marked using biotinylated nucleotides. Subsequently, 3C templates are fragmented and enriched for 3C-fragments carrying a biotin tag and, thus, representing hybrid molecules informative for a given 3D-chromatin contact. After ligation of adapter sequences to the fragmented 3C templates, the entire pool of enriched fragments is sequenced using high-throughput sequencing technology. A number of Hi-C variants have been established, varying the number of cells used as input material [36], the digestion procedure [29], and the enrichment [27] strategy.

Obtaining a genome-wide 3D contact map, typically represented by a contact frequency matrix, requires elaborate computational data processing and also significant computational resources. Various tools which preprocess and analyze complex Hi-C data are available today (see website cited above); however, to be able to fully understand and explore the obtained data, skills in computational biology are still required. Furthermore, faithful calling of 3D-contacts relies on the quality of the respective reference genome. Flaws in reference genomes can easily lead to artifacts, whose misinterpretation may lead to wrong conclusions. Hence, previous experience in analyzing genomics data represents an advisable prerequisite for successful application of Hi-C technology.

The obtained sequencing data is of high complexity, proportional to the square of the number of primary restriction fragments found in the entire genome. Small genomes, such as the one of *Drosophila melanogaster* or *Arabidopsis thaliana* contain approximately 30,000 fragments (given a six-cutter restriction enzyme), which yield a combinatorial space of nearly one billion contacts. Hence, achieving fragment size resolution requires considerable sequencing depth. Larger genomes, such as mammalian genomes and certain plant genomes, require exponentially more sequencing depth, and, hence, achieving fine-grain resolution using Hi-C may be challenging and cost-intensive.

Hi-C may appear as the gold-standard technology of the 3C family and, indeed, it is an extremely powerful technique not only to characterize global chromosomal architecture, but also to reveal previously unsuspected chromosomal contacts of biological significance. However, despite its power and relative ease to perform (especially compared to 3C and 5C), considerable limitations have to be taken into consideration. Identification of enhancer–promoter interactions may be challenging using Hi-C, as the required resolution can only be achieved with considerable investment in sequencing depth. Hence, although perfectly suited to acquire a global comprehension of 3D organization, investigations on

specific chromosomal contacts maybe easier conducted using 3C or 4C.

Hi-C represents of the most widely used methods of the 3C collection in plants. In several studies, Hi-C has been used to characterize general chromosomal architecture [37–39] and specific chromosomal 3D conformations, such as the *KNOT*, positive strips [39] and chromatin loops [40]. Furthermore, the effects of various mutants on chromosomal architecture have been assessed by Hi-C [37, 38, 41, 42]. These studies provided a better understanding of the folding principles of the Arabidopsis genome and, importantly, revealed previously unknown genomic features, which can be related to gene regulation [40] and genome defense mechanisms [38].

1.5 Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) and HiChIP: Protein-Centered Chromatin Interaction Approaches

- *Type*: All-to-all (bound by the same protein factor).
- *Application*: Detection of chromatin contacts, mediated by specific proteins.
- *Protocols*: [43–45].

ChIA-PET [46] resembles Hi-C technology, as it allows to detect genome-wide chromosomal contact frequencies (*see* Fig. 1). However, ChiA-PET does not represent a candidate-free approach, as chromatin contacts mediated by a protein factor of interest are enriched during the experimental protocol with the help of immunoprecipitation. Hence, a protein-specific interactome can be generated. Using this technique, it was shown that human estrogen receptor α (ER-α) mediates long-range contacts between regulatory ER-α binding sites and target gene promoters [46].

The ChIA-PET procedure significantly differs from all other experimental protocols of the 3C family. Cross-linked chromatin is physically fragmented by sonication and genomic fragments bound by a given protein factor are immunoprecipitated using specific antibodies. Linker sequences are ligated to the free DNA ends of the DNA–protein complexes. These linkers are subsequently integrated during proximity ligation that will create hybrid DNA molecules, consisting of protein factor bound genomic regions. With the help of the integrated linker sequences paired-end tag sequencing is performed to identify and quantify protein factor-mediated chromatin contacts. Due to the enrichment of a small set of genomic regions and enzyme-free fragmentation of the chromatin, ChIA-PET can yield a high resolution, allowing the pinpointing of biologically relevant chromatin interactions.

To date, ChIA-PET technology has not been as widely used as other 3C methods. However, in the presence of strong candidate protein factors, ChiA-PET represents a powerful method to identify genome-wide chromatin contacts of biological relevance.

Recently, another protein centered approach, termed HiChIP, has been established, which promises both higher yield of informative sequencing reads and lower requirements for the input material [45]. Whereas ChiA-PET requires up to 100 million cells, HiChIP has been reported to provide comparable output with as little as 1 million cells. HiChIP methodology shares more similarities to the other 3C members: In a first step, biotinylated Hi-C templates are generated. These templates are subsequently sheared by sonication followed by an immunoprecipitation with an antibody against the protein factor of interest. All downstream steps, including library generation, generally follow the standard Hi-C protocol. Computational pipelines to specifically analyze HiChIP are not implemented to date. However, standard Hi-C analysis programs are compatible with HiChIP data and may be sufficient to answer a wide range of research questions.

Hence, HiChIP promises to replace ChIA-PET in a foreseeable future, as it may generate more informative data output and, importantly, involves less complex methodology. Due to its novelty, independent experts of the field could not yet confirm the reliability of HiChIP. Thus, potential pitfalls of HiChIP may come to light with more researchers performing HiChIP.

HiChIP and ChIA-PET are promising methods to unravel fundamental questions on plant 3D chromatin organization. Thereby, a special focus could be laid on large regulatory protein complexes, such as Polycomb group (PcG) complexes. PcG complexes may play an important role in shaping 3D interactions and, hence, may represent an obvious target in order to establish HiChIP in plants.

1.6 Capture Hi-C (CHi-C)/Targeted Chromatin Capture (T2C)

- *Type*: Many-to-many/Many-to-all.
- *Application*: Determination of chromatin contacts of a specific set of chromosomal regions.
- *Protocols*: [47–50].

CHi-C [47, 49, 50] and T2C [48] technologies are recent members of the 3C collection. They allow identifying genome-wide chromosomal contacts of a specific set of genomic loci of interest, such as poised and active promoters with genome-wide interactors of those regions (*see* Fig. 1). Capture Hi-C methods are based on generation of a canonical 3C library; however, genomic regions of interest are enriched employing various capture sequencing strategies, such as the use of biotinylated RNA bait libraries. Using this strategy, capture Hi-C methods can either generate data similar to 5C, yielding a detailed description of chromosomal contacts within a genomic region of interest, or data similar to multiple 4C experiments, simultaneously characterizing the genome-wide interaction profiles of a large number of viewpoints (*see* Fig. 3). Hence, major

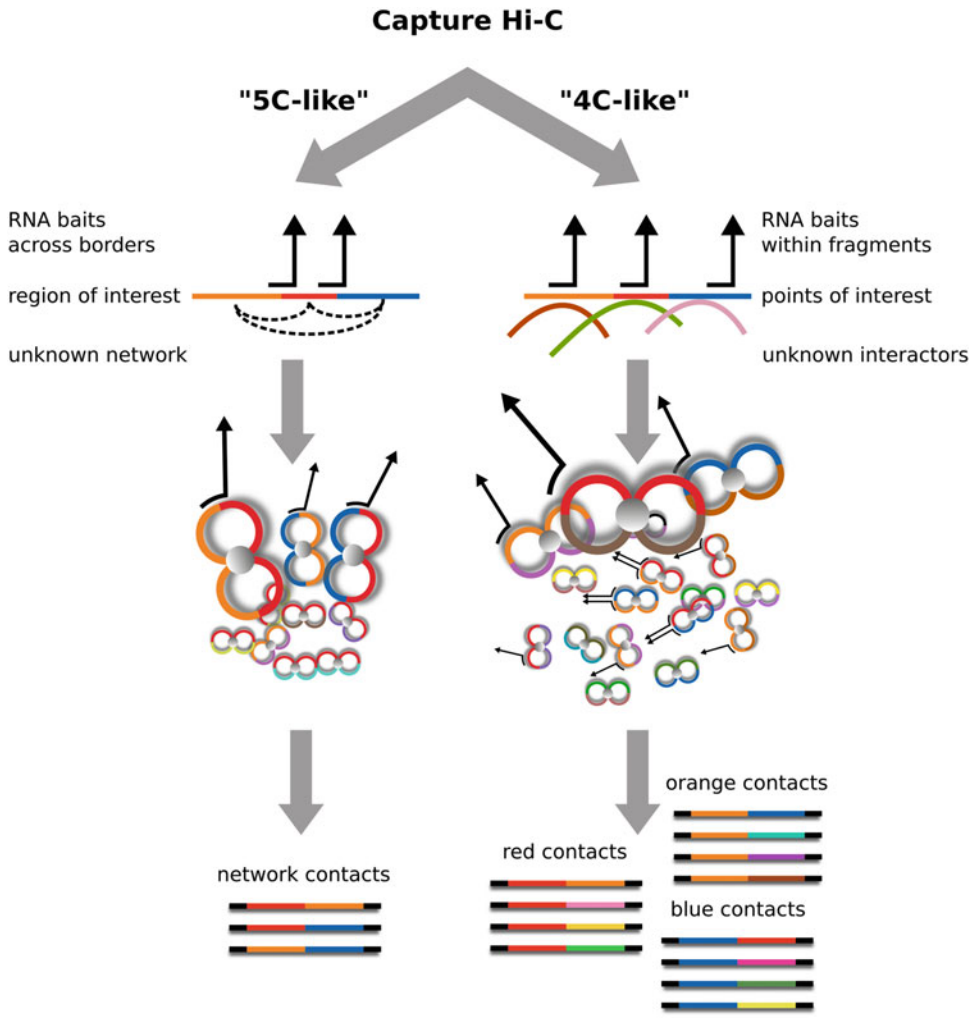

Fig. 3 Applications of Capture Hi-C. Capture Hi-C can generate two separate experimental outputs, depending on the design of biotinylated RNA baits. By probing single fragments, multiple "4C-like" datasets can be generated. By designing RNA baits across fragment borders, 5C like data can be generated, allowing the characterization of interaction networks of chromosomal regions of interest

limitations of Hi-C are overcome, namely, the high complexity of Hi-C libraries, which renders it difficult or very cost intensive to achieve high resolution. Furthermore, yielding a similar experimental output as 5C and multiple 4C, it eliminates the necessity to generate a large set of primers, which is not only cost-intensive but also a latent source of experimental biases, due to imbalanced PCR amplification efficiency.

For various research questions, a candidate-free approach is not required; hence, Capture Hi-C offers impressive resolution and limits amplification steps, which are a constant source of experimental noise that needs to be tightly controlled. As all candidate

approaches however, it does not allow detection of previously unanticipated chromosomal contacts.

Related to 5C in their experimental output, CHi-C and T2C may be employed to answer a variety of biological questions, ranging from identification of potential promoter–enhancer interactions to detailed and unbiased characterization of 3D structuring of large genomic loci.

2 Conclusion

The collection of 3C technologies is highly proliferative, leading to the appearance of novel 3C-based approaches nearly every year over the last decade. Hence, even for experts of the field, it proves to be difficult to follow up on the technological advances and to assess the pros and cons of different 3C variants for specific research questions. However, with the current set of 3C variants at hand, we are finally offered a wide range of opportunities to study chromosomal architecture from various angles. These rapid technological advances are currently leading to a flourishing research field, which has been previously received insufficient attention, caused by technical inaccessibility of 3D chromatin structures. Hence, in recent years we rapidly gained a deepened understanding of plant 3D genome organization. Most of the published studies employing 3C technology in plants, to date, are of descriptive nature, setting a base line for future work (reviewed in [51, 52]). This work could now be aimed at understanding how the 3D genome influences fundamental cellular processes, such as transcription, differentiation, genome defense, and, last but least, genome evolution.

3C technologies represent the key to explore a fascinating level of genome organization. This may not only serve to more precisely define previously studied biological processes, but may lead to the discovery of novel biological phenomena as well.

References

1. Hagège H, Klous P, Braem C et al (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). Nat Protoc 2:1722–1733. doi:10.1038/nprot.2007.243
2. Louwers M, Splinter E, van Driel R et al (2009) Studying physical chromatin interactions in plants using Chromosome Conformation Capture (3C). Nat Protoc 4:1216–1229. doi:10.1038/nprot.2009.113
3. Miele A, Dekker J (2008) Mapping cis-and trans-chromatin interaction networks using chromosome conformation capture (3C). In: Hancock R. (ed) The nucleus: volume 2: chromatin, transcription, envelope, proteins, dynamics, and imaging. Methods in molecular biology. Humana, Totowa 464:105–121. doi:10.1007/978-1-60327-461-6_7
4. Dekker J (2006) The three "C" s of chromosome conformation capture: controls, controls, controls. Nat Methods 3:17–21. doi:10.1038/nmeth823
5. Comet I, Schuettengruber B, Sexton T, Cavalli G (2011) A chromatin insulator driving three-dimensional Polycomb response element (PRE) contacts and Polycomb association with the chromatin fiber. Proc Natl Acad Sci U S A 108:2294–2299. doi:10.1073/pnas.1002059108

6. Weber B, Jamge S, Stam M (2017) 3C analysis in Maize and Arabidopsis. Methods Mol Biol
7. Dekker J (2002) Capturing Chromosome Conformation. Science 295:1306–1311. doi:10.1126/science.1067799
8. Louwers M, Bader R, Haring M et al (2009) Tissue- and expression level-specific chromatin looping at Maize b1 Epialleles. Plant Cell 21:832–842. doi:10.1105/tpc.108.064329
9. Crevillen P, Sonmez C, Wu Z, Dean C (2012) A gene loop containing the floral repressor FLC is disrupted in the early phase of vernalization. EMBO J 32:140–148. doi:10.1038/emboj.2012.324
10. Ariel F, Jegu T, Latrasse D et al (2014) Noncoding transcription by alternative RNA polymerases dynamically regulates an auxin-driven chromatin loop. Mol Cell 55:383–396. doi:10.1016/j.molcel.2014.06.011
11. Dostie J, Zhan Y, Dekker J (2007) Chromosome conformation capture carbon copy technology. Curr Protoc Mol Biol Chapter 21:Unit 21.14. doi: 10.1002/0471142727.mb2114s80
12. Dostie J, Dekker J (2007) Mapping networks of physical interactions between genomic elements using 5C technology. Nat Protoc 2:988–1002. doi:10.1038/nprot.2007.116
13. Lajoie BR, van Berkum NL, Sanyal A, Dekker J (2009) My5C: web tools for chromosome conformation capture studies. Nat Methods 6:690–691. doi:10.1038/nmeth1009-690
14. Dostie J, Richmond TA, Arnaout 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. doi:10.1101/gr.5571506
15. Sauria ME, Phillips-Cremins JE, Corces VG, Taylor J (2015) HiFive: a tool suite for easy and efficient HiC and 5C data analysis. Genome Biol:1–10. doi:10.1186/s13059-015-0806-y
16. Simonis M, Klous P, Homminga I et al (2009) High-resolution identification of balanced and complex chromosomal rearrangements by 4C technology. Nat Methods 6:837–842. doi:10.1038/nmeth.1391
17. Grob S, Schmid MW, Luedtke NW et al (2013) Characterization of chromosomal architecture in Arabidopsis by chromosome conformation capture. Genome Biol 14:R129. doi:10.1186/gb-2013-14-11-r129
18. Sexton T, Kurukuti S, Mitchell JA et al (2012) Sensitive detection of chromatin coassociations using enhanced chromosome conformation capture on chip. Nat Protoc 7:1335–1350. doi:10.1038/nprot.2012.071
19. Grob S (2017) Circular chromosome conformation capture in plants. Methods Mol Biol 1610:73–92. doi:10.1007/978-1-4939-7003-2_6
20. Zhao Z, Tavoosidana G, Sjölinder M et al (2006) Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat Genet 38:1341–1347. doi:10.1038/ng1891
21. 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. doi:10.1038/ng1896
22. van de Werken HJG, Landan G, Holwerda SJB et al (2012) Robust 4C-seq data analysis to screen for regulatory DNA interactions. Nat Methods 9:969–972. doi:10.1038/nmeth.2173
23. Klein FA, Pakozdi T, Anders S et al (2015) FourCSeq: analysis of 4C sequencing data. Bioinformatics 31:3085–3091. doi:10.1093/bioinformatics/btv335
24. Robinson MD, McCarthy DJ, Smyth GK (2009) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. doi:10.1093/bioinformatics/btp616
25. Grob S, Grossniklaus U (2017) Chromatin Conformation Capture-based analysis of nuclear architecture. Methods Mol Biol 1456:15–32. doi: 10.1007/978-1-4899-7708-3_2
26. van Berkum NL, Lieberman-Aiden E, Williams L et al (2010) Hi-C: a method to study the three-dimensional architecture of genomes. J Vis Exp:1–7. doi:10.3791/1869
27. Kalhor R, Tjong H, Jayathilaka N et al (2011) Genome architectures revealed by tethered chromosome conformation capture and population-based modeling. Nat Biotechnol 30:90–98. doi:10.1038/nbt.2057
28. Belton J-M, McCord RP, Gibcus JH et al (2012) Hi-C: a comprehensive technique to capture the conformation of genomes. Methods 58:268–276. doi:10.1016/j.ymeth.2012.05.001
29. Ramani V, Cusanovich DA, Hause RJ et al (2016) Mapping 3D genome architecture through in situ DNase Hi-C. Nat Protoc 11:2104–2121. doi:10.1038/nprot.2016.126
30. 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. doi:10.1126/science.1181369

31. Sexton T, Yaffe E, Kenigsberg E et al (2012) Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 148:458–472. doi:10.1016/j.cell.2012.01.010
32. Korbel JO, Lee C (2013) Genome assembly and haplotyping with Hi-C. Nat Biotechnol 31:1099–1101. doi:10.1038/nbt.2764
33. Kaplan N, Dekker J (2013) High-throughput genome scaffolding from in vivo DNA interaction frequency. Nat Biotechnol 31:1143–1147. doi:10.1038/nbt.2768
34. Adey A, Patwardhan RP, Qiu R et al (2013) Chromosome-scale scaffolding of de novo genome assemblies based on chromatin interactions. Nat Biotechnol 31:1119–1125. doi:10.1038/nbt.2727
35. Lesne A, Riposo J, Roger P et al (2014) 3D genome reconstruction from chromosomal contacts. Nat Methods 11:1141–1143. doi:10.1038/nmeth.3104
36. Nagano T, Lubling Y, Stevens TJ et al (2013) Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature 502:59–64. doi:10.1038/nature12593
37. Feng S, Cokus SJ, Schubert V et al (2014) Genome-wide Hi-C analyses in wild-type and mutants reveal high-resolution chromatin interactions in Arabidopsis. Mol Cell 55:694–707. doi:10.1016/j.molcel.2014.07.008
38. Grob S, Schmid MW, Grossniklaus U (2014) Hi-C analysis in Arabidopsis identifies the KNOT, a structure with similarities to the flamenco locus of Drosophila. Mol Cell 55:678–693. doi:10.1016/j.molcel.2014.07.009
39. Wang C, Liu C, Roqueiro D et al (2014) Genome-wide analysis of local chromatin packing in Arabidopsis thaliana. Genome Res 25:246–256. doi:10.1101/gr.170332.113
40. Liu C, Wang C, Wang G et al (2016) Genome-wide analysis of chromatin packing in Arabidopsis thaliana at single-gene resolution. Genome Res 26:1057–1068. doi:10.1101/gr.204032.116
41. Moissiard G, Cokus SJ, Cary J et al (2012) MORC family ATPases required for heterochromatin condensation and gene silencing. Science 336:1448–1451. doi:10.1126/science.1221472
42. Veluchamy A, Jegu T, Ariel F et al (2016) LHP1 regulates H3K27me3 spreading and shapes the three-dimensional conformation of the Arabidopsis Genome. PLoS One 11: e0158936–e0158925. doi:10.1371/journal.pone.0158936
43. Fullwood MJ, Han Y, Wei C-L et al (2010) Chromatin interaction analysis using paired-end tag sequencing. Curr Protoc Mol Biol Chapter 21:Unit 21.15.1–25. doi: 10.1002/0471142727.mb2115s89
44. Goh Y, Fullwood MJ, Poh HM et al (2012) Chromatin interaction analysis with paired-end tag sequencing (ChIA-PET) for mapping chromatin interactions and understanding transcription regulation. J Vis Exp. doi: 10.3791/3770
45. Mumbach MR, Rubin AJ, Flynn RA et al (2016) HiChIP: efficient and sensitive analysis of protein-directed genome architecture. Nat Methods 13:919–922. doi:10.1038/nmeth.3999
46. Fullwood MJ, Liu MH, Pan YF et al (2009) An oestrogen-receptor-α-bound human chromatin interactome. Nature 461:58–64. doi:10.1038/nature08497
47. Dryden NH, Broome LR, Dudbridge F et al (2014) Unbiased analysis of potential targets of breast cancer susceptibility loci by Capture Hi-C. Genome Res 24:1854–1868. doi:10.1101/gr.175034.114
48. Kolovos P, van de Werken HJ, Kepper N et al (2014) Targeted Chromatin Capture (T2C): a novel high resolution high throughput method to detect genomic interactions and regulatory elements. Epigenetics Chromatin 7:10–17. doi:10.1186/1756-8935-7-10
49. Mifsud B, Tavares-Cadete F, Young AN et al (2015) Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat Genet 47:598–606. doi:10.1038/ng.3286
50. Jäger R, Migliorini G, Henrion M et al (2015) Capture Hi-C identifies the chromatin interactome of colorectal cancer risk loci. Nat Commun 6:6178. doi:10.1038/ncomms7178
51. Liu C, Weigel D (2015) Chromatin in 3D: progress and prospects for plants. Genome Biol 16:1–6. doi:10.1186/s13059-015-0738-6
52. Grob S, Grossniklaus U (2017) Chromosome Conformation Capture-based studies reveal novel features of plant nuclear architecture. Curr Opin Plant Biol 36:149–157