Chromosome conformation capture with deep sequencing to study the roles of the Structural Maintenance of Chromosomes complex *in vivo*

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ABSTRACT

Recent applications of chromosome conformation capture with deep sequencing (Hi-C and other C techniques) has enabled high throughput investigations and driven major advances in understanding chromosome organization in bacteria and eukaryotes. C techniques reveal systematically the identities of interacting DNA and the frequency of each interaction *in vivo*. Beyond a bird's-eye view survey of the global chromosome architecture, C techniques together with genetic perturbation have proven to be powerful in understanding factors that shape chromosome architectures. The Structural Maintenance of Chromosomes (SMC) proteins play major roles in organizing the chromosomes from bacteria to humans, and C techniques have contributed to understanding their mechanism and impact on genome organization in a cellular context. Here, I describe a Hi-C protocol, a variant of C techniques, to construct genome-wide DNA contact maps for bacteria. This protocol is optimized for the Gram-negative bacterium *Caulobacter crescentus*, but it can be readily adapted for any bacterial species of interest.

Keywords: chromosome conformation capture, Hi-C, deep sequencing, chromosome organization, Structural Maintenance of Chromosomes, SMC, *Caulobacter crescentus*.

Running title: Characterizing the roles of bacterial SMC by Hi-C

1 INTRODUCTION

The Structural Maintenance of Chromosomes (SMC) proteins are highly conserved from bacteria to humans. They regulate nearly all aspects of chromosome biology [1–6]. In eukaryotes, SMC1/3 together with non-SMC accessory proteins form a cohesin complex that is required for the establishment and maintenance of sister-chromatid cohesion until all sister chromatids achieve bipolar attachment to the mitotic spindle. Therefore, cohesin is crucial for a proper chromosome segregation. On the other hand, condensin complex (SMC2/4 with accessory proteins) is required for chromosome condensation during mitosis. Both cohesin and condensin also have crucial functions in regulating gene expression. Lastly, SMC5/6 complex has multiple roles in DNA damage repair. In evolutionary terms, SMCs are of bacterial origin, and yet the function of bacterial SMC is less well studied than the function of its eukaryotic counterparts. Much like eukaryotic chromosomes, bacterial chromosomes cannot be packed haphazardly. Instead, they must be organized and adopt structures that are compatible with chromosome replication, chromosome segregation, DNA damage repair, and gene expression regulation [7, 8]. Although molecular insight into the structure and mechanism of bacterial SMC has been gained *in vitro* [9–12], our understanding of its mechanism and impact on genome organization in a cellular context is still limited.

Recent applications of chromosome conformation capture with deep sequencing (Hi-C) (*see* Fig. 1) has enabled a high throughput investigation and driven advances in understanding chromosome organization in bacteria and eukaryotes [13–26]. Hi-C reveals systematically the identities of interacting DNA and the frequency of each interaction *in vivo* [13]. We and others have applied Hi-C to various bacterial species to reveal the global organization of their chromosomes *in vivo* [19, 21–24]. The first application of Hi-C to bacteria examined the *Caulobacter crescentus* chromosome [19, 27]. Hi-C analysis confirmed the global pattern of chromosome organization in *Caulobacter*: in cells with a single chromosome, the origin of replication (*ori*) and the terminus (*ter*) are at opposite cell poles, and the two chromosomal arms are well-aligned, running in parallel down the long axis of the cell [19, 28] (*see* Fig. 2). Hi-C analysis showed that *Caulobacter* and *Bacillus subtilis* lacking SMC has reduced interactions between opposite chromosomal arms, suggesting a role of SMC in chromosome organization, potentially by actively tethering the two chromosome arms [17, 18] (*see* Fig. 2). Hi-C and genetic perturbation have proven to be powerful in investigating the molecular mechanism of SMC *in vivo*.

Here, I describe an optimized Hi-C protocol to generate genome-wide DNA contact maps for *Caulobacter*. *Caulobacter* is well suited for Hi-C analysis because the cells are easily synchronized [29], enabling us to generate genome-wide data for a homogenous population of G1-phase cells that each contain a single chromosome. As there is no active DNA replication in the G1 cells, the effect of transcription on SMC translocation and global chromosome organization can be isolated and studied, without confounding effects from replication-transcription conflicts [18]. However, Hi-C is applicable to a wide-range of bacterial species, and this protocol here can be readily adapted for any bacterial species of interest, without the need for synchronization (*see* **Note** 1). We also recommend that researchers consult other excellent protocols and reviews on C techniques in bacteria and eukaryotes to be best informed about critical parameters such as the choice of restriction enzymes, crosslinking conditions, and sequencing depth before embarking on optimizing Hi-C for your species of interest [13, 30–36]. For *in silico* analysis of Hi-C data, we routinely use and adapt scripts from the Mirny lab to analyze bacterial Hi-C data [19, 24, 37] (see **Note** 2). Computational analysis of C data is outside the scope of this methods article, hence is not covered here.

2 MATERIALS

2.1 Culture fixation

1. 1 x M2 salts buffer: 6.1 mM Na_2HPO_4, 3.9 mM KH_2PO_4, 9.3 mM NH_4Cl, 0.5 mM $MgSO_4$, 10 µM $FeSO_4$, and 0.5 mM $CaCl_2$
2. 0.5 mL and 1.5 mL standard microcentrifuge tubes
3. 36.5% (v/v) formaldehyde solution
4. 2.5 M glycine solution: weigh 46.8 g of glycine and transfer to a 500 mL beaker. Add ultrapure water to a volume of 250 mL, and dissolve glycine using a magnetic stirrer. Apply gentle heating to facilitate the dissolution of glycine. Filter the solution on a 0.22 µm filtering unit. The solution is stable for a month at room temperature (RT).

2.2 Hi-C library construction

1. Ready-Lyse lysozyme
2. 50,000 units/mL *Bgl*II restriction enzyme
3. 10,000 units/mL *Cla*I restriction enzyme
4. Restriction enzyme buffer 2 and 3
5. 2,000,000 units/mL T4 DNA ligase
6. 10 x T4 ligase buffer
7. 3,000 units/mL T4 DNA polymerase
8. 10,000 units/mL T4 polynucleotide kinase
9. 5,000 units/mL Klenow large fragment
10. 5,000 units/mL Klenow 3'-5' exo^-
11. 2 mM dGTP, 2mM dTTP, and 2mM dCTP
12. 0.4 mM biotin-14-dATP
13. 20 mg/mL proteinase K solution
14. 5% (w/v) sodium dodecyl sulfate (SDS)
15. 10% (v/v) Triton X-100
16. 10 mg/mL bovine serum albumin (BSA)
17. 15 mg/mL GlycoBlue co-precipitant
18. 0.25 M EDTA solution pH 8.0
19. 3 M sodium acetate solution pH 5.2
20. 25:24:1 phenol:chloroform:isoamyl alcohol pH 8.0
21. 100% isopropanol
22. 1 x TE buffer pH 8.0: 10 mM Tris-HCl pH 8.0 and 1mM EDTA
23. 1 x NTB buffer: 5 mM Tris-HCl pH 8.0, 0.5 mM EDTA, and 1M NaCl
24. 0.5 mL and 1.5 mL standard and LoBind microcentrifuge tubes
25. 0.2 mL PCR tubes
26. Magnetic rack
27. Water baths at 10°C, 25°C, 37°C, and 65°C
28. Refrigerated benchtop centrifuges
29. Bioruptor sonication device for DNA shearing (Diagenode)
30. 1.5 mL TPX microcentrifuge tubes and adaptor (Diagenode)

2.3 Illumina sequencing library construction

1. 400,000 units/mL T4 DNA ligase
2. MinElute Reaction CleanUp columns
3. Qiaquick Gel Extraction columns
4. 1% and 2% agarose gel
5. 1xTAE buffer for agarose gel electrophoresis
6. NEBNext Multiplex Oligos kit for Illumina that includes NEBNext Adaptor, USER (Uracil-Specific Excision Reagent) enzyme, NEBNext Universal PCR Primer, and NEBNext Index Primers.
7. DynaBead MyOne Streptavidin C1
8. Phusion polymerase enzyme, 100% DMSO, 5x HF buffer, and 10 mM dNTP for polymerase chain reaction (PCR)
9. Thermocycler

3 METHODS

The general scheme for Hi-C library construction is summarized in Fig. 1. Hi-C technique utilizes formaldehyde to crosslink protein-DNA and DNA-DNA to preserve the chromosome conformation. Proximity ligation is then used to join DNA fragments together. The ligated junctions containing information on which DNA loci are interacting together *in vivo* are then pulled down and subjected to deep sequencing. Two important sets of information are retrieved: (i) the sequence identities of interacting DNA, and (ii) the frequencies of their interactions. It is worth remembering that Hi-C (and other C techniques) measures interaction frequencies, not physical distances between DNA loci.

The preparation of Hi-C libraries can take two to three days, and the generation of Illumina sequencing libraries take an additional day, depending on the number of libraries being processed in parallel. I have indicated below when reactions can be safely stopped and stored without affecting the quality of the libraries. We routinely prepare four Hi-C libraries in parallel. I do not recommend handling more than ten Hi-C samples at the same time.

3.1 Culture fixation

1. Incubate *Caulobacter* cells at OD_{600} of 0.2 with formaldehyde (final concentration of 1%) in the culturing broth with gentle shaking (*see* **Note** 3). Formaldehyde crosslinks protein-DNA and DNA-DNA together, thereby capturing the structure of the chromosome at the time of fixation (*see* Fig. 1). Allow the crosslinking reaction to proceed for 30 min at RT.
2. Add 2.5 M glycine to a final concentration of 0.125 M, and incubate with gentle shaking for 15 min at RT to quench the fixation by formaldehyde.
3. Pellet fixed cells by centrifugation (10,000 x g for 10 min, at 4°C) and discard the supernatant.
4. Wash the fixed cells twice with 1 x M2 salts buffer before resuspending them in an appropriate volume of 1 x TE buffer to a final concentration of 10^7 cells/µL (*see* **Note** 4).
5. Divide the resuspended cells into 25 µL aliquots and store them individually in 0.5 mL microcentrifuge tubes at -80°C for no more than four weeks.

3.2 Hi-C library construction

6. Two 25 µL aliquots of the same sample are routinely used for each Hi-C experiment. Add 0.25 µL of Ready-Lyse lysozyme to each 25 µL cell aliquot, mix gently by pipetting up and down several times, and incubate for 15 min at RT.
7. Add 1.25 µL of 5% SDS to the lysozyme-treated cells aliquot in **step 6**, mix gently by pipetting up and down several times, and incubate for a further 15 min at RT to completely dissolve cell membranes and to release chromosomal DNA (*see* **Note** 5).
8. Add 5 µL of restriction enzyme buffer 3, 5 µL of 10% Triton X-100, and 11 µL of autoclaved ultrapure water to the reaction from **step 7**. Mix gently by pipetting up and down several times, and incubate for 15 min at RT (*see* **Note** 6).
9. Add 2.5 µL of 50,000 units/mL *Bgl*II restriction enzyme, mix gently by pipetting, and incubate at 37°C for 3 hours to digest the chromosomal DNA (Fig. 1B) (*see* **Note** 7).
10. Cool the reaction on ice before proceeding to label sticky ends with biotin-14-dATP (Fig. 1B). Assemble the following reaction: 50 µL of restriction enzyme digestion mix from **step 9**, 0.9 µL of 2mM dGTP, 0.9 µL of 2mM dTTP, 0.9 µL of 2mM dCTP, 4.5 µL of 0.4 mM biotin-14-dATP, 1.6 µL of autoclaved ultrapure water, and 1.2 µL of Klenow large fragment.
11. Incubate for 45 min at RT before adding 3 µL of 5% SDS to stop the reaction.
12. In this step, filled-in DNA ends are ligated together in a dilute condition so that DNA fragments that were spatially close *in vivo* and fixed together by formaldehyde would be preferably ligated together while ligation between randomly colliding DNA fragments in the microcentrifuge tube is minimized (*see* Fig. 1). Prepare the ligation buffer consisting of 75 µL of 10% Triton X-100, 100 µL of 10 x T4 ligation buffer, 5 µL of 10 mg/mL BSA, and 800 µL of autoclaved ultrapure water in a 1.5 mL microcentrifuge tube. Mix all the components well by inverting the tube several times and leave on ice for 15 min.
13. Mix the content of the labeling reaction in **step 11** with the ligation buffer in a 1.5 mL microcentrifuge tube, and leave at RT for at least 15 min (*see* **Note** 8).
14. Transfer the microcentrifuge tube back on ice for at least 15 min before proceeding to the next step.
15. Add 3 µL of concentrated T4 DNA ligase (2,000,000 units/mL) to the ligation reaction,

mix all the components well, and incubate at 10°C for 5 hours (*see* **Note** 9).

16. Add 40 µL of 0.25 M EDTA pH 8.0 to stop the ligation reaction, mix all the components well by inverting the tube several times.
17. Add 2.5 µL of 20 mg/mL proteinase K, mix all the components well by inverting the tube several times.
18. Incubate the reaction in a 65°C water bath overnight to reverse crosslinks and remove bound proteins.
19. In the next day, extract DNA from **step 18** twice with phenol/chloroform/isoamyl alcohol pH 8.0, precipitate DNA using isopropanol with the help of the GlycoBlue co-precipitant, and finally dry and resuspend the DNA pellet in 60 µL of water (*see* **Note** 10). The purified DNA can be safely stored at -20°C after this step.
20. In this step, unligated but biotin-labeled fragments are eliminated using the 3'-5' exonuclease activity of T4 DNA polymerase. Assemble the following reaction in a 0.2 mL PCR tube: 60 µL of purified DNA from **step 19**, 10 µL of restriction enzyme buffer 2, 1 µL of 10 mg/mL BSA, 5 µL of 2 mM dGTP, 23.5 µL of water, and 0.5 µL of T4 DNA polymerase. Incubate the reaction at 12°C for 2 hours. Use a thermocycler set at 12°C to maintain an accurate temperature.
21. Extract DNA using phenol/chloroform/isoamyl alcohol pH 8.0, precipitate DNA using isopropanol and GlycoBlue co-precipitant, and finally dry and resuspend the DNA pellet in 100 µL of water (*see* **Note** 10). The purified DNA can be safely stored at -20°C after this step.
22. Transfer 100 µL of purified DNA to a 1.5 mL TPX microcentrifuge tubes for shearing in the Bioruptor sonication device (Fig. 1). Shear DNA to 200 bp-500 bp fragments using the Bioruptor sonicator (*see* **Note** 11).
23. Electrophorese the fragmented DNA on a 2% agarose gel. Excise the gel band containing DNA between 200 bp and 500 bp, and purify DNA from the agarose using gel extraction columns (*see* **Note** 12). Elute the DNA with 50 µL of autoclaved water. The purified DNA can be safely stored at -20°C after this step.

3.3 Illumina sequencing library construction

24. From this step onwards, LoBind microcentrifuge tubes are used to minimize DNA loss. End-repair DNA in a reaction consisting of: 50 µL of sheared Hi-C DNA from **step 23**, 10 µL of 10 x T4 DNA ligase buffer, 2.5 µL of 10 mM dNTPs, 28.75 µL of water, 4 µL of T4 DNA polymerase, 4 µL of T4 polynucleotide kinase, 0.75 µL of Klenow large fragment. Mix all the components well by pipetting up and down several times, and incubate at 25°C for 30 min (*see* **Note** 13).
25. Purify DNA using MinElute Reaction CleanUp columns, and elute DNA with 30 µL of water (*see* **Note** 14).
26. Attach A-overhangs to the 3' ends of the repaired DNA by incubating 30 µL of DNA from **step 25** with 4 µL of 10 x restriction enzyme buffer 2, 4 µL of 2 mM dATP, and 3 µL of Klenow 3'-5' exo$^-$. Mix all the components well by pipetting up and down several times, incubate the reaction at 37°C for 45 min.
27. Purify DNA again using MinElute Reaction CleanUp columns, and elute DNA with 15 µL of water.
28. Ligate purified DNA from **step 27** with the NEBNext adaptor in the following reaction: 15 µL of DNA from **step 27**, 5 µL of NEBNext adaptor, 2.5 µL of 10 x T4 ligase buffer, 1 µL of water, and 1.5 µL of T4 ligase enzyme (400,000 units/mL). Mix all the components well and incubate the reaction at 25°C for 30 min (*see* **Note** 15).
29. Add 1 µL of USER enzyme, mix gently by pipetting up and down several times, and incubate the reaction at 37°C for 15 min to process the NEBNext adaptor.
30. In this step, biotin-labeled DNA are purified away from non-labeled DNA using DynaBead MyOne Streptavidin C1 beads (*see* Fig. 1). Wash 25 µL of Streptavidin C1 beads in 200 µL of NTB buffer twice by repeating a cycle of resuspension and pull-down by magnetic attraction.
31. Transfer the washed beads to the ligation mixture in **step 29**, and incubate with a gentle agitation at RT for 30 min to capture the biotin-labeled DNA.
32. Pull down beads using a magnetic rack and discard the unwanted supernatant.
33. Wash beads from **step 32** twice in 200 µL of NTB buffer, twice in 200 µL of water, and finally resuspend the beads in 10 µL of water.
34. Enrich DNA bound on beads by PCR using primers compatible with Illumina paired-end sequencing chemistry. Assemble the following PCR reaction: 1 µL of NEBNext Universal PCR primer, 1 µL of NEBNext Index primer, 1 µL of 10mM dNTP, 10 µL of 5

x HF buffer, 1.5 µL of 100% DMSO, 35 µL of water, 0.5 µL of Phusion DNA polymerase enzyme, and 1.2 µL of the resuspended beads from **step 33** (*see* **Note** 16).

35. Amplify DNA using the following PCR program: 30 sec at 98ºC, (10 sec at 98°C, 20 sec at 60°C, 25 s at 72 °C) x 14 cycles, 5 min at 72ºC, 5 min at 4ºC.
36. Purify PCR products by gel extraction before sequencing on Illumina HiSeq platforms (*see* **Note** 17).

4 NOTES

1. Without synchronization of cell cultures, the background signal of DNA contact maps are likely to be high. However, most of the constant features of the chromosome are still observable [17, 21, 22, 24].
2. Toolboxes and scripts for analysis of *Caulobacter* Hi-C data can be found at: https://bitbucket.org/mirnylab/hiclib. We also recommend that researchers consult other excellent reviews and protocols on computational analysis of C data here [31, 38].
3. The concentration of formaldehyde is optimized empirically for each species of interest. We routinely use 1% formaldehyde to fix *Caulobacter* cells for Hi-C experiments [19], other research groups use 3% to 5% formaldehyde to fix cultures of *Bacillus subtilis* or *Escherichia coli* for Hi-C/3C-seq experiments [22–24].
4. One mL of *Caulobacter* culture at OD_{600} of 0.1 contains approximately 10^8 cells.
5. A gentle mixing by pipetting slowly up and down several times is recommended to minimize mechanical shearing of chromosomal DNA. It should be easy to pipette up and down, and the solution should not be viscous if the fixation of the cell culture was done adequately.
6. This is a critical step: incubate the reaction for 15 min to allow adequate time for Triton X-100 to inactivate SDS from **step 7**.
7. The choice of restriction enzymes and digestion buffers is critical for the success of Hi-C experiments. Different restriction enzymes have different restriction frequency, depending on the distributions of their recognition sites on bacterial genomes. This distribution determines the theoretical resolution of Hi-C contact maps. For *Caulobacter crescentus*, we routinely use *Bgl*II which gives a 10-kb resolution for Hi-C contact maps. Beyond the issue of resolution, many restriction enzymes do not cut optimally in the bacterial cell lysate. Some of the proven restriction enzymes for Hi-C experiments are *Bgl*II, *Hind*III, *Eco*RI, and *Nco*I. Note that we use a highly concentrated *Bgl*II enzyme in this step (*see* **Materials**). We recommend that researchers determine the efficiency of restriction enzyme digestion by agarose gel electrophoresis (*see* [30] for an excellent review on quality controls for C libraries in *Caulobacter crescentus*).

8. This is a critical step: incubate for at least 15 min to allow adequate time for Triton X-100 to inactivate SDS from **step 12**.
9. We use a highly-concentrated T4 DNA ligase in this step (*see* **Materials**). Also, we do not add extra ATP to the ligation reaction as the 10 x T4 DNA ligase buffer (NEB) is already supplemented with 1 mM ATP. The final concentration of *Caulobacter* chromosomal DNA in the ligation mix is estimated to be ~0.5 ng/µL i.e. lower than the concentration used in the previous 3C study in yeast (~2.5 ng/µL) [39]. Given that the *Caulobacter* genome is ~3 times smaller than that of yeast, the lower concentration of *Caulobacter* DNA used in a ligation reaction gives a comparable low background of random ligation products.
10. We precipitate DNA using 100% ice-cold isopropanol instead of ethanol to avoid handling a large volume of solvent. One volume of isopropanol per volume of aqueous DNA solution, instead of three volume of ethanol, is required for DNA precipitation. Also, we recommend researchers to check the integrity of DNA and the efficiency of ligation (**step 15**) by 1% agarose gel electrophoresis after DNA has been precipitated and resolubilized here. The presence of a relatively tight band of high molecular weight (greater than 10 kb if *Bgl*II was used in **step 9**) indicates a good ligation. We also recommend performing PCR or qPCR to confirm the abundance of a positive-control Hi-C junction (*see* [30] for an excellent review on quality controls for C libraries in *Caulobacter crescentus*). The amplified Hi-C junction is resistant to digestion by *Bgl*II but susceptible to restriction by *Cla*I (*see* Fig. 1B). The *Cla*I restriction site can be used to assess biotin-dATP fill-in efficiency.
11. Other DNA shearing devices can be used to fragment the DNA. We routinely use a sonication setting of 30 sec on, 30 sec off, for 30 min to achieve a desired fragmentation on the Bioruptor device. We recommend that researchers optimize the sonicator settings empirically for each instrument. For the Bioruptor device, the use of hard-plastic 1.5 mL TPX microcentrifuge tubes and exactly 100 µL of DNA solution ensures a consistent fragmentation of DNA.
12. We include RNaseA in the loading dye for agarose gel electrophoresis, this eliminates any residual RNA that co-precipitates with DNA in previous steps. We recommend that excised gel bands are dissolved in the gel extraction buffer by vortexing. Avoid the use of high heat to dissolve the agarose gel band.
13. Commercial kits can be adapted to construct Hi-C Illumina sequencing libraries. However, we find the traditional method of preparing sequencing libraries that uses individual enzymes results in a much higher yield.
14. MinElute Reaction CleanUp columns are used to maximize the recovery of eluted DNA.
15. We use the ready-made NEBNext adaptor (*see* **Materials**) to construct Hi-C sequencing libraries. This necessitates the use of a USER enzyme (*see* **Materials**) to further process the adaptor (**step 29**). If home-made adaptors or adaptors from a different commercial company are used, skip **step 29** or modify accordingly.
16. This is a specific PCR protocol to amplify DNA from *Caulobacter* since DNA from this organism is high in G+C content. Modify this program to suit the bacterial species of interest. Use a different NEBNext Index primer for each different Hi-C sample, this allows samples to be barcoded, pooled, and sequenced on the same Illumina sequencing lane.
17. Gel extraction to purify PCR product is preferred over size-selection beads as we can remove nearly all unwanted Illumina adaptor dimers. We routinely pool five to ten barcoded samples for each Illumina HiSeq 2500 sequencing lane. Given the small size of *Caulobacter* genome (~4.2 Mb) and that Hi-C junctions are enriched by biotin labeling and streptavidin pull-down, 10 million of raw paired-end sequencing reads are sufficient to generate a genome-wide Hi-C contact map at the resolution of ~10 kb. After *in silico* filtering of unligated and PCR duplicated DNA fragments, researchers should expect more than 5 million informative reads for the construction of Hi-C contact map. If the fraction of informative reads is significantly less than 50% of the total sequencing reads, it is an indication of a sub-optimal Hi-C experiment. We recommend that researcher check **step 1**, **step 12**, or **step 20** again.

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Fig. 1

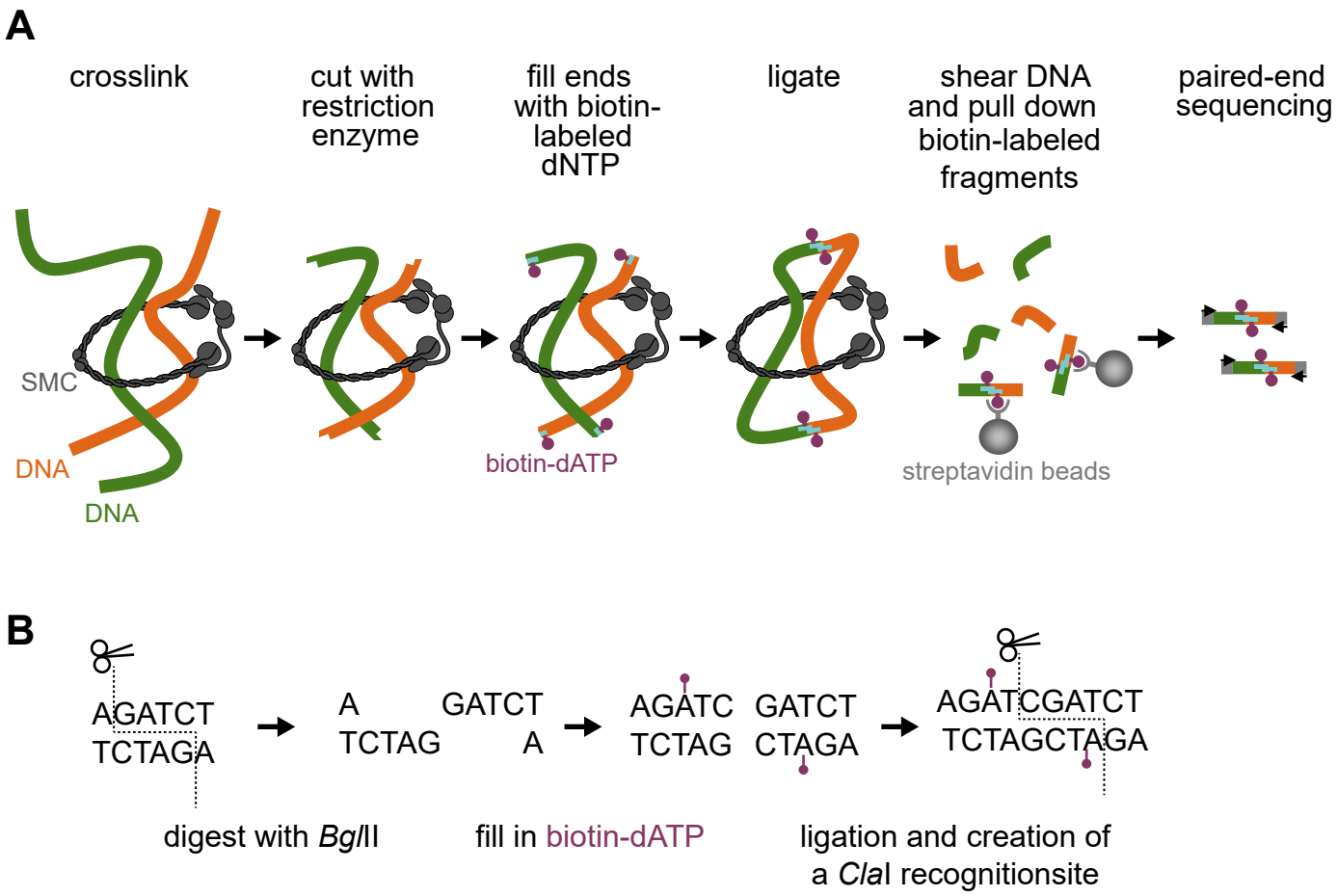

Fig. 1. A scheme for the chromosome conformation capture with deep sequencing (Hi-C). (a) Hi-C technique utilizes formaldehyde to crosslink protein-DNA and DNA-DNA to preserve the chromosome conformation. Chromosomal DNA is digested with restriction enzyme, and proximity ligation is then used to join DNA fragments together. The ligated junctions containing information on which DNA loci are interacting together *in vivo* are then pulled down and subjected to deep sequencing. In this schematic picture, SMC is depicted as a generic protein that binds DNA together. Note that formaldehyde indiscriminately crosslinks any DNA-binding proteins to their DNA. **(b)** Digestion of chromosomal DNA by *Bgl*II, fill in sticky ends with biotin-dATP, ligation and the creation of the *Cla*I recognition site at the ligated Hi-C junction. The *Cla*I restriction site can be used to assess fill-in efficiency.

Fig. 2

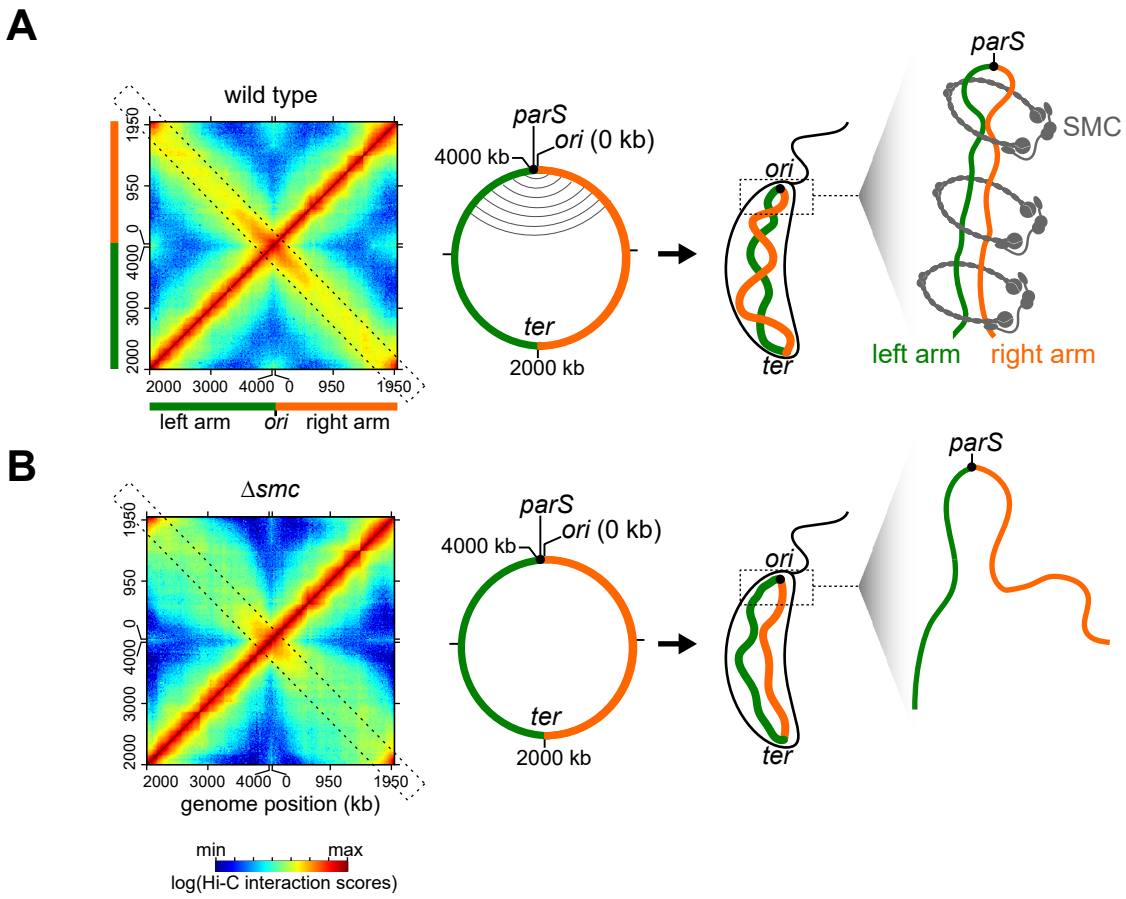

Fig. 2. Hi-C combined with genetic perturbation revealed a possible mechanism of SMC in *Caulobacter crescentus*. Normalized Hi-C contact maps use colors to indicate the frequency of interactions between locus pairs across the genome. The secondary diagonal (dashed box) indicates interactions between opposite chromosome arms in **(a)** wild-type *Caulobacter* cells and in **(b)** cells lacking SMC. Cells lacking SMC show a dramatic reduction in inter-arm DNA-DNA interactions, suggesting a role of SMC in promoting interactions between chromosome arms in *Caulobacter*. A simplified genomic map of *Caulobacter* shows the origin of replication (*ori*), the *parS* site, and the terminus (*ter*), together with left (green) and the right (orange) chromosomal arms. On the genomic map of wild-type cells, DNA regions aligned by SMC are presented schematically as grey curved lines connecting the two chromosome arms. It is unclear whether SMC can hold both chromosome arms within its lumen or two SMC, each encircles a chromosome arm can handcuff to tether both arms together. For simplicity, only SMC encircling both arms is shown schematically. Pictures are not to scale.