Protocol

Hi-C in Budding Yeast

Jon-Matthew Belton and Job Dekker¹

Program in Systems Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Hi-C enables simultaneous detection of interaction frequencies between all possible pairs of restriction fragments in the genome. The Hi-C method is based on chromosome conformation capture (3C), which uses formaldehyde cross-linking to fix chromatin regions that interact in three-dimensional space, irrespective of their genomic locations. In the Hi-C protocol described here, cross-linked chromatin is digested with HindIII and the ends are filled in with a nucleotide mix containing biotinylated dCTP. These fragments are ligated together, and the resulting chimeric molecules are purified and sheared to reduce length. Finally, biotinylated ligation junctions are pulled down with streptavidin-coated beads, linked to high-throughput sequencing adaptors, and amplified via polymerase chain reaction (PCR). The resolution of the Hi-C data set will depend on the depth of sequencing and choice of restriction enzyme. When sufficient sequence reads are obtained, information on chromatin interactions and chromosome conformation can be derived at single restriction fragment resolution for complete genomes.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Agarose

AmPure XP magnetic bead-based purification system (Beckman Coulter)

The AmPure XP mixture is used to fractionate the library into 100- to 300-bp fragments for high-throughput sequencing.

ATP (100 mM)

Binding buffer (BB) (1× and 2×) <R>

Bovine serum albumin (BSA) (10 mg/mL)

Buffer EB, prewarmed to 65°C (QIAGEN 19086)

DNA polymerase I, large (Klenow) fragment (5 U/μL) (New England Biolabs M0210S)

DNA quantification reagents for the method of choice (e.g., Bioanalyzer, qPCR, or fluorometry)

Dynabeads MyOne Streptavidin C1 beads (Life Technologies 65001)

Ethanol (100% and 70%)

Formaldehyde (37%) (Fisher Scientific BP531-500)

¹Correspondence: job.dekker@umassmed.edu

© 2015 Cold Spring Harbor Laboratory Press

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot085209

Glycine (2.5 M, filter-sterilized)

Klenow Fragment (3'→5' exo-) (5 U/μL) (New England Biolabs M0212S)

Ligation buffer for 3C (10×) <R>

NEBuffer 2 (New England Biolabs B7002S) (10× and 1×)

Nucleotides

Biotin-14-dCTP (0.4 mM) (Life Technologies 19518-018)

dATP (100 mM and 1 mM)

dGTP (100 mM)

dTTP (100 mM)

Nucleotide (dNTP) mix (25 mM per nucleotide; 100 mM total)

Nucleotide (dNTP) mix (1.25 mM per nucleotide; 5 mM total)

Oligonucleotide primers

Hi5: GTTTCCGAAAATCCACGACGAACCAG (80 μM)

Hi6: ATATTTTCGCCGAGGTGCTGGAAAT (80 μM)

PE1.0:

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
(HPLC-purified) (25 μM)

PE2.0:

CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCC
GATCT (HPLC-purified) (25 μM)

Paired-end adapters compatible with the Illumina HiSeq or GAII platforms (available from Illumina, Kapa Biosystems, or Bioo Scientific)

PfuUltra II Fusion HS polymerase with 10× reaction buffer (Agilent Technologies 600850)

Phase Lock Gel Light tubes (15-mL) (5-Prime 2302840)

Phase Lock Gel Light tubes (50-mL) (5-Prime 2302860)

Phenol:chloroform (1:1) <R>

Proteinase K (Life Technologies 25530-015)

Prepare a solution of 10 mg/mL in TE buffer <R>.

Quick Ligation Kit (Quick T4 DNA ligase and 2× Quick Ligation Reaction Buffer) (New England Biolabs M2200S)

Restriction enzymes

HindIII (20,000 U/mL) (New England Biolabs R0104S)

NheI (10,000 U/mL) (New England Biolabs R0131S)

RNase A, DNase-free (10 mg/mL)

Saccharomyces cerevisiae

Grow yeast cells (using growth medium and conditions appropriate for the strain and experiment) for 2–3 doubling times to obtain mid-log phase cells. Usually, a 100-mL culture produces enough cells to generate one Hi-C library.

Sodium acetate (3 M, pH 5.2) <R>

Sodium dodecyl sulfate (SDS) (10% and 1% [w/v])

Standards for gel electrophoresis (e.g., 1-kb ladder and low molecular mass DNA ladder
[New England Biolabs N3232S and N3233S])

T4 DNA ligase (1 U/μL) (Life Technologies 15224-017)

T4 DNA ligase reaction buffer (10×) (New England Biolabs B0202S)

T4 DNA polymerase (3 U/μL) (New England Biolabs M0203L)

T4 polynucleotide kinase (10 U/μL) (New England Biolabs M0201S)

TBE buffer <R>

TE buffer <R>

TLE buffer <R>
Triton X-100 (10% [v/v])
Tween wash buffer (TWB) <R>

Equipment

AFA microTUBES with snap caps (Covaris 520045)
Centrifugal filters (Amicon 15-mL, 30 kDa) (Millipore UFC903024)
Centrifugal filters (Amicon 500-μL, 30 kDa) (Millipore UFC5030BK)
Centrifuge (high-speed, refrigerated, for up to 18,000g)
Centrifuge (tabletop, refrigerated, for up to 3100g)
Densitometer
DNA LoBind tubes (1.7-mL)
DNA quantification equipment for the method of choice (e.g., Bioanalyzer, qPCR, or fluorometry)
Dry ice
Flask (250-mL)
Gel electrophoresis apparatus
Incubator at 37°C, with rotating platform
Incubator/shaker at 25°C
Liquid nitrogen
Magnetic particle separator (MPS)
Microcentrifuge tubes (1.7-mL)
MinElute columns (QIAGEN 28004)
Mortar and pestle

Immediately before use, precool a mortar and pestle by placing them on dry ice and adding enough liquid nitrogen to the mortar to cover the head of the pestle. Let the liquid nitrogen evaporate before use (Step 9).

PCR tubes (0.2-mL)
Rotator
Sonicator (Covaris S2)

The Covaris S2 is the sonicator of choice since it shears the DNA to 50–700 bp fragments in only 4 min. (Any other sonicator will also work, but the exact conditions and setting must be determined empirically for naked DNA, and it may not produce as tight of a smear.) Fill the water chamber of the sonicator with deionized water and allow it to cool down and degas for at least 30 min before sonication (Step 73).

Spectrometer to measure optical density at 600 nm
Thermal cycler
Thermomixer or water baths at 16°C, 20°C, 37°C, 65°C, and 75°C
Tubes, conical (15- and 50-mL)
Tubes, screw cap (35- and 250-mL, suitable for high-speed centrifugation)
Vacuum aspirator
Vortex

METHOD

An overview of Hi-C is provided in Figure 1.

Cross-Linking Chromatin

Because the output of Hi-C is the rate of cross-linking, it is essential to standardize cross-linking across all samples.

1. Add 37% formaldehyde to the cultured yeast cells to a final concentration of 3% of the culture medium (i.e., 8.82 mL of 37% formaldehyde per 100-mL culture).
2. Shake the culture at 200 rpm for 20 min at 25°C.
3. Quench the cross-linking by adding 2.5 M glycine at 2× the volume of formaldehyde used in Step 1 (i.e., 17.64 mL of 2.5 M glycine per 100 mL of culture).

FIGURE 1. Schematic overview of the Hi-C procedure and quality control. (A). Hi-C is based on 3C, in which the chromatin is cross-linked using formaldehyde and digested with a restriction enzyme (see Protocol: **Chromosome Conformation Capture (3C) in Budding Yeast** [Belton and Dekker 2015]). Hi-C is unique in that the overhangs of the restriction cut site are filled in with a biotinylated nucleotide before ligation. Intra-molecular ligation and purification are very similar to the 3C protocol; however, the Hi-C method contains a few additional steps to enrich for ligation products. First, biotin incorporated into free ends (not at a ligation junction of two different fragments) is removed using the exonuclease activity of T4 DNA polymerase. The library is then fragmented by sonication and true ligation products are enriched by pull-down using streptavidin-coated beads. (B) The purified Hi-C library runs at ~12 kb on a 0.8% agarose gel (Step 65), indicated by the arrow. M, 1-kb DNA ladder. (C) Agarose gel for estimation of Hi-C efficiency (Step 68). H, HindIII digested; N, NheI digested; B, both HindIII and NheI digested; O, no digestion of the PCR product; M, low molecular mass marker. The *upper* arrow indicates the undigested PCR product and the two *lower* arrows indicate the digestion products. (D) Electrophoresis of PCR cycle titration (Step 110). M, low molecular mass marker; “6,” “9,” “12,” “15,” “18” are the number of cycles used to amplify the library. The *bottom* arrow indicates the correct size of the Hi-C library and the *upper* arrow indicates high-molecular-mass species that arise from overamplifying the library.

4. Shake the culture at 200 rpm for 5 min at 25°C.
5. Transfer the cells to a 250-mL tube and centrifuge the cells at 1800g for 5 min in a tabletop centrifuge.
6. Pour off the medium and wash the cells in 100 mL of sterile H₂O by pipetting up and down until the cell pellet is resuspended.
7. Centrifuge the cells at 1800g for 5 min in a tabletop centrifuge.
8. Pour off the supernatant and resuspend the cells in 5 mL of 1× NEBuffer 2 by pipetting up and down.
9. Add liquid nitrogen to a prechilled mortar and pour the sample into the liquid nitrogen. Once the sample has frozen, begin to crush it with the pestle. When the sample is broken into little pieces, begin to grind it with the pestle. Grind the sample for 10 min, adding liquid nitrogen as necessary (approximately every 3 min).

10. Scrape the sample into a 50-mL tube on ice. Add 45 mL of ice-cold 1× NEBuffer 2 to the lysate.
11. Centrifuge the lysate at 1800g for 5 min at 4°C in a tabletop centrifuge.
12. Pour off the supernatant and resuspend the cells in 1× NEBuffer 2 to an OD₆₀₀ of 10.0.
The lysate can be stored in 7.2-mL aliquots at −80°C for several years.

Digesting Cross-Linked Chromatin with HindIII

13. If frozen, thaw one 7.2-mL aliquot of lysate on ice. Transfer the lysate to a 50-mL tube and wash with 50 mL of ice-cold 1× NEBuffer 2 by inverting the tube several times.
14. Centrifuge the lysate at 3100g for 10 min at 4°C in a tabletop centrifuge.
15. Aspirate the supernatant with a vacuum. Resuspend the pellet in 5.5 mL of 1× NEBuffer 2.
16. Distribute 456 µL of cell lysate into each of twelve 1.7-mL tubes.
17. Solubilize the chromatin by adding 45.6 µL of 1% SDS per tube. Mix by pipetting up and down. Resuspend any precipitated cellular debris, but avoid making bubbles.
18. Incubate the tubes for 10 min at 65°C. Place the tubes on ice immediately after the incubation.
It is critical to place the samples on ice after this incubation, as high temperature reverses formaldehyde cross-links.
19. Centrifuge the tubes for 10 sec to remove liquid from the tube caps.
20. Quench the SDS by adding 52.8 µL of 10% Triton X-100 per tube. Mix by pipetting up and down. Resuspend any precipitated cellular debris, but avoid making bubbles.
21. Digest the chromatin by adding 60 µL of HindIII (20,000 units/mL) per tube. Mix by pipetting up and down. Resuspend any precipitated cellular debris, but avoid making bubbles.
22. Incubate the tubes overnight at 37°C.
It is preferable to agitate the sample during incubation, so a rotating platform in a 37°C incubator is recommended.

Incorporating Biotin at Digested Ends

23. Centrifuge the tubes for 10 sec to remove liquid from the tube caps.
24. Add the following reagents to each tube.

NEBuffer 2 (10×)	6.4 µL
dATP (100 mM)	0.18 µL
dGTP (100 mM)	0.18 µL
dTTP (100 mM)	0.18 µL
Biotin-14-dCTP (0.4 mM)	45.0 µL
DNA polymerase I (Klenow) (5 U/µL)	12.0 µL
25. Incubate all reactions for 2 h at 37°C.
It is preferable to agitate the sample during the incubation, so a rotating platform in a 37°C incubator is recommended.
26. Centrifuge the tubes for 10 sec to remove liquid from the tube caps.
27. Denature the enzyme by adding 115.2 µL of 10% SDS to each tube. Mix by pipetting up and down but avoid making bubbles.
28. Incubate the tubes for 20 min at 65°C. Place the tubes on ice immediately after the incubation.

Ligating Cross-Linked Chromatin Fragments

Below, chromatin fragments are ligated in a dilute reaction to favor intramolecular ligation. Because variations in ligation conditions can contribute to variation in background ligation (intermolecular ligation) and reduce the signal-to-noise ratio, it is essential to standardize intra-molecular ligation across all samples.

29. Assemble the ligation master mix by adding the following to a 250-mL flask on ice. Mix the reaction by gently swirling but avoid making bubbles.

Triton X-100 (10%)	13.0 mL
Ligation buffer for 3C (10×)	13.0 mL
BSA (10 mg/mL)	1.4 mL
ATP (100 mM)	1.4 mL
H ₂ O (sterile)	104 mL
T4 DNA ligase (1 U/μL)	3.51 mL

30. Add 10.5 mL of ligation master mix to each of twelve 15-mL tubes on ice.
31. Centrifuge the tubes for 10 sec to remove the liquid from the caps.
32. Using a 1-mL micropipette, transfer each Hi-C reaction from Step 28 to a 15-mL tube containing ligation master mix. Mix the tubes gently by inverting.
33. Incubate the ligation reactions for 8 h at 16°C. Invert the tubes every hour.

Reversing Cross-Links and Purifying Ligation Products

34. Add 72 μL of proteinase K (10 mg/mL) to each ligation reaction from Step 33. Mix by inverting. Incubate reactions at 65°C overnight.
35. Add another 72 μL of proteinase K (10 mg/mL) to each reaction. Mix by inverting. Incubate reactions for 2 h at 65°C.
36. Transfer each reaction to a 50-mL tube. Add 23.0 mL of phenol:chloroform (1:1) to each tube and vortex each tube for 30 sec.
37. Pour each vortexed sample into a prespun 50-mL tube of Phase Lock Gel Light. Centrifuge at 1500g for 10 min.
38. Pour the aqueous phase of each sample into a fresh 50-mL tube. Add 23.0 mL of phenol:chloroform (1:1) to each tube and vortex each tube for 30 sec.
39. Pour each vortexed sample into a prespun 50-mL tube of Phase Lock Gel Light. Centrifuge at 1500g for 10 min.
40. Pool the aqueous phases from all samples into two 250-mL tubes (~67.8 mL/tube).
41. Precipitate the 3C ligation products by adding 1/10th volumes (6.78 mL) of 3 M sodium acetate (pH 5.2) and 2.5× volumes (169.5 mL) of 100% ethanol to each tube.
42. Incubate the tubes on dry ice for 30–45 min. Make sure the liquid is very cold and thick but not frozen.
43. Centrifuge the tubes at 10,000g for 20 min at 4°C.
44. Carefully pour off the supernatant and discard. Centrifuge at 10,000g for 30 sec at 4°C to collect the remaining drops of liquid at the bottom of the tube.
45. Aspirate the remaining alcohol using a vacuum.
46. Resuspend both Hi-C DNA pellets in 4 mL total of TE buffer and transfer the sample to a 15-mL tube.
47. Add 8.0 mL of phenol:chloroform (1:1) to the Hi-C sample and vortex for 30 sec.
48. Transfer the sample to a prespun 15-mL tube of Phase Lock Gel Light. Centrifuge in a tabletop centrifuge at 1500g for 10 min.
49. Transfer the aqueous phase to a fresh 15-mL tube. Add 8 mL of phenol:chloroform (1:1) and vortex the tube for 30 sec.
50. Transfer the sample to a prespun 15-mL tube of Phase Lock Gel Light. Centrifuge in a tabletop centrifuge at 1500g for 10 min.

51. Transfer the aqueous phase to a 35-mL tube. Precipitate the 3C DNA with 400 μ L of sodium acetate (pH 5.2) and 10.0 mL of 100% ethanol.
52. Centrifuge the sample at 18,000g for 20 min.
53. Decant the supernatant and centrifuge the sample at 10,000g for 30 sec at 4°C to collect remaining liquid.
54. Aspirate the remaining alcohol using a vacuum and allow the DNA pellet to air dry.
55. Resuspend the Hi-C sample in 15 mL of TE and vortex for 60 sec.
56. Transfer the sample to a 15-mL Amicon 30-kDa filter column for desalting.
57. Centrifuge the sample in a tabletop centrifuge at 3100g for 15 min.
58. Discard the flowthrough and wash the sample with 15 mL of TE. Repeat the centrifugation in Step 57.
59. Elute the Hi-C DNA from the 15-mL filter by pipetting the remaining sample out of the filter with a 200- μ L micropipette. Transfer the sample to a 500- μ L Amicon 30 kDa filter column.
60. Wash the surface of the 15-mL filter with 200 μ L of TE. Pipette the 200 μ L off the filter and add this to the rest of the sample in the 500- μ L filter column.
61. Centrifuge the sample in the 500- μ L filter column at 18,000g for 20 min. If the volume in the column is >20 μ L, centrifuge repeatedly for 5 min at a time until it is <20 μ L.
62. Elute the Hi-C sample by inverting the column and placing it in a fresh collection tube. Centrifuge the tube at 18,000g for 20 min.
63. Adjust the sample volume to 20 μ L with TE.
64. Degrade any co-precipitated RNA by adding 2 μ L of RNase A (10 mg/mL). Incubate the sample for 1 h at 37°C.
65. Perform gel electrophoresis of dilutions of the library DNA (0.25 to 2.0 μ L) on a 0.8% agarose gel in 0.5 $\times$ TBE. Quantify the DNA by comparison to known concentration standards (500 to 100 ng).

The Hi-C library DNA should appear as a tight band ~12 kb on the gel (Fig. 1B). Any smearing of the sample may be caused by overly vigorous lysis or the presence of endogenous nucleases in the strain.

The typical yield of a Hi-C library is 3–10 μ g, with an average yield of ~5 μ g.

Estimating Hi-C Efficiency

Hi-C efficiency is the percentage of ligation products for a single, neighboring interaction (an interaction between restriction fragments that are adjacent to one another in the genome) that are biotinylated. This value is used to standardize the amount of streptavidin beads used to enrich the library for biotinylated ligation products and gives a measure of the efficiency of the biotin fill-in.

66. Use 40 ng of the Hi-C library to PCR-amplify a neighboring interaction as follows. Place the rest of the Hi-C library DNA at 4°C until ready to proceed with Step 69.

- i. Prepare the reaction by combining the following reagents in a 0.2-mL PCR tube.

Hi-C library DNA from Step 65	40 ng
PfuUltra II reaction buffer (10 $\times$)	10 μ L
dNTPs (100 mM; 25 mM each nucleotide)	0.8 μ L
Primer Hi5 (80 μ M)	0.5 μ L
Primer Hi6 (80 μ M)	0.5 μ L
PfuUltra II polymerase	2.0 μ L
H ₂ O (sterile)	to 100 μ L

- ii. Amplify the ligation product of interest using the following program on a thermal cycler: 5 min at 95°C; 35 cycles of 30 sec at 95°C, 30 sec at 65°C, and 30 sec at 72°C; and 8 min at 72°C.

67. Digest the amplicon with HindIII, NheI, and HindIII/NheI as follows.
 - i. Prepare the HindIII digestion containing 15 μ L of PCR product, 1.9 μ L of 10 $\times$ NEBuffer 2, 0.19 μ L of BSA, 0.95 μ L of HindIII (20,000 units/mL), and 0.95 μ L of sterile H₂O.
 - ii. Prepare the NheI digestion containing 15 μ L of PCR product, 1.9 μ L of 10 $\times$ NEBuffer 2, 0.19 μ L of BSA, 0.95 μ L of NheI, and 0.95 μ L of sterile H₂O.
 - iii. Prepare the double-digest containing 15 μ L of PCR product, 1.9 μ L of 10 $\times$ NEBuffer 2, 0.19 μ L of BSA, 0.95 μ L of HindIII, and 0.95 μ L of NheI.
 - iv. Prepare a control containing 15 μ L of PCR product, 1.9 μ L of 10 $\times$ NEBuffer 2, 0.19 μ L of BSA, and 1.9 μ L of sterile H₂O.
 - v. Incubate the digestion reactions overnight at 37°C.
68. Perform gel electrophoresis of the digested and undigested bands on a 2% agarose gel in 0.5 $\times$ TBE (Fig. 1C). Quantify the bands by densitometry, and estimate the Hi-C efficiency.

Not all PCR products will be cut in the NheI and HindIII combined reaction. This is likely due to inefficiency of digestion and point mutations introduced during amplification, and may also be caused by DNA breakage during the procedure leading to noncanonical ligation products. Thus, the Hi-C efficiency, which is the percentage of digested products in the NheI digestion, should be corrected by the percent cleavable, which is determined from the combined reaction. The final Hi-C efficiency is therefore calculated as the percent digested with NheI divided by the percent digested with NheI and HindIII. Generally, 30%–60% of the amplicon will digest with NheI.

See Troubleshooting.

Removing Biotin from Free Ends

Proceed with the entire Hi-C sample (set aside in Step 66) throughout the rest of the protocol.

69. For each 1 μ g of Hi-C library DNA, add the following reagents to the tube of DNA: 0.1 μ L of BSA (10 mg/mL), 1.0 μ L of 10 $\times$ NEBuffer 2, 0.1 μ L of 5 mM dNTPs (1.25 mM per nucleotide), and 1.0 μ L of T4 DNA polymerase (3 U/ μ L). Adjust the final volume to 10.0 μ L with sterile H₂O.
70. Incubate the reaction for 4 h at 20°C and then for 20 min at 75°C to inactivate the enzyme.
71. Adjust the volume to 102 μ L by adding sterile H₂O or concentrating the sample using a 500- μ L 30-kDa Amicon filter as appropriate.

Shearing the Hi-C Library

72. Load 101 μ L of the Hi-C sample into an AFA microTUBE. Set aside 1.0 μ L of unsonicated DNA for electrophoresis analysis in Step 95.
73. Place the tube into the Covaris-approved S2 holder and run the following program: Duty Cycle, 10%; Intensity, 5; Cycles per Burst, 200; Mode, Frequency sweeping. Continue to degas during the sonication. Run the program for a total of 4 min. Set aside 1.0 μ L of sonicated DNA for electrophoresis analysis in Step 95.
74. Purify the DNA using one MinElute column for every 5 μ g of Hi-C reaction. Elute the DNA from each column using 31 μ L of prewarmed (65°C) Buffer EB.

Repairing DNA Ends and A-Tailing

75. Add the following reagents to each of the sonicated elutions from Step 74.

T4 DNA Ligase Reaction Buffer (10 $\times$)	14.0 μ L
dNTPs (100 mM; 25 mM each)	1.4 μ L
T4 DNA polymerase (3 U/ μ L)	5.0 μ L
T4 polynucleotide kinase (10 U/ μ L)	5.0 μ L
DNA polymerase I (Klenow) (5 U/ μ L)	1.0 μ L
H ₂ O (sterile)	13.6 μ L

76. Incubate the reactions for 30 min at 20°C.
77. Purify the DNA using one MinElute column per reaction. Elute each sample with 31 µL of prewarmed (65°C) Buffer EB.
78. Add the following reagents to each of the end-repaired samples.

NEBuffer 2 (10×)	5.0 µL
dATP (1.0 mM)	10.0 µL
Klenow fragment (<i>exo-</i>) (5 U/µL)	3.0 µL
H ₂ O (sterile)	2.0 µL
79. Incubate the reactions for 30 min at 37°C.
80. Inactivate the enzyme by incubating the reactions for 20 min at 65°C.

Fractionating the Sonicated Hi-C Library

81. Pool all end-repaired samples from Step 80 and adjust the volume to 500 µL with Buffer EB.
82. Add 450 µL of AMPure XP to the Hi-C sample. Label this sample “0.9X.”
83. Vortex the mixture briefly and then quickly centrifuge to remove liquid from the tube cap. Incubate at room temperature for 10 min.

Proceed to Step 84 during this incubation.
84. While the “0.9X” sample is incubating, prepare another tube (“1.1X”) containing AmPure XP as follows.
 - i. Add 500 µL of AmPure XP to a new 1.7-mL tube and label this tube “1.1X”.
 - ii. Place the “1.1X” tube on an MPS for 5 min to collect the AmPure XP beads to the side of the tube.
 - iii. While the tube is still on the MPS, remove and discard the AmPure solution using a 1-mL micropipette.
 - iv. Resuspend the collected beads in 100 µL of Ampure XP.

This process increases the number of beads in the Ampure XP mixture and ensures there is enough binding capacity to collect all of the DNA in the Hi-C sample.
85. Place the “0.9X” sample on an MPS for 5 min to collect the AmPure XP beads to the side of the tube.
86. While still on the MPS, transfer the supernatant from the “0.9X” sample to the “1.1X” tube using a 1-mL micropipette.

Retain the “0.9X” beads for washing in Step 90.
87. Vortex the “1.1X” sample briefly and then quickly centrifuge to remove liquid from the cap. Incubate the mixture at room temperature for 10 min.
88. Place the “1.1X” sample on an MPS for 5 min to collect the Ampure XP beads to the side of the tube.
89. While still on the MPS, remove and discard the AmPure solution from the sample using a 1-mL micropipette.
90. Wash the “0.9X” and “1.1X” beads twice with 200 µL of 70% ethanol per wash as follows.
 - i. Resuspend the beads in 70% ethanol and centrifuge the tube quickly.
 - ii. Collect the beads on an MPS.
 - iii. Discard the ethanol.
91. After the second ethanol wash, centrifuge the sample again and collect any remaining ethanol. Air-dry the beads at room temperature.

92. Resuspend the “0.9X” and the “1.1X” beads in 30 μ L of prewarmed (65°C) Buffer EB per tube. Elute the DNA at room temperature for 10 min.
93. Place the “0.9X” and the “1.1X” tubes on an MPS for 5 min to collect the AmPure XP beads to the side of each tube.
94. While still on the MPS, transfer the AmPure solution from each tube to a separate 1.7-mL tubes using a 1-mL micropipette.
The “0.9X” sample contains DNA fragments >300 bp and the “1.1X” sample contains DNA fragments between 100 bp and 300 bp. The “1.1X” sample, which contains the portion of the library that is compatible with the Illumina platform, will be used for all subsequent steps.
95. Perform gel electrophoresis of 1.0 μ L of each of the unsonicated (Step 72), sonicated (Step 73), “0.9X” and “1.1X” samples on a short, 2% agarose gel in 0.5X TBE at 250 volts for 30 min. Verify the size of the 1.1X fraction and quantify it by comparison to a known concentration standard (10.0 to 100 ng).

Enriching Biotinylated Ligation Products

96. Calculate the quantity of biotinylated ligation product in the library as follows.
 - i. Multiply the amount of DNA (ng) in the 1.1X fraction, measured in Step 95, by 200 (the average length of the sonicated Hi-C library). Divide this amount by 8000 (the average length of the presonication library).
This quantity is an estimate of the amount of the sample that contains a ligation product.
 - ii. Multiply the quantity from Step 96.i by the proportion of biotinylated ligation products (Hi-C efficiency) calculated in Step 68.
This is the quantity of biotinylated ligation product in the library. This is used to calculate the amount of Dynabeads MyOne Streptavidin C1 beads to use for the pull down.
97. For each 1.0 ng of biotinylated ligation product calculated in Step 96, add 1.0 μ L of Dynabeads MyOne Streptavidin C1 beads to a 1.7-mL LoBind tube.
LoBind tubes are used in this step to reduce nonspecific pull-down of DNA.
98. Wash the beads twice with TWB as follows.
 - i. Resuspend the beads in the wash buffer using a micropipette.
 - ii. Transfer the beads to a new LoBind tube and incubate for 3 min with rotation.
 - iii. Quickly centrifuge the beads to the bottom of the tube.
 - iv. Reclaim the beads on an MPS for 1 min.
99. Resuspend the beads in 30 μ L of 2 $\times$ BB.
100. Add the 1.1X fraction from Step 94 to the beads and incubate for 1 h at room temperature with rotation.
101. Reclaim the beads on an MPS for 1 min.
The supernatant should be retained as insurance until the end of the protocol.
102. Wash the beads once with 200 μ L of 1 $\times$ BB as described in Steps 98.i–98.iv.
103. Wash the beads once with 50 μ L of 1 $\times$ NEB Quick Ligation Buffer as described in Steps 98.i–98.iv.
104. Resuspend the beads in 10 μ L of sterile H₂O.
105. Ligate the Illumina PE adapters to the biotinylated products by adding 13 μ L of 2 $\times$ Quick Ligation Reaction Buffer, 2 μ L of Illumina PE adapters and 1 μ L of Quick T4 DNA Ligase to the beads. Incubate the ligation reaction at room temperature for 15 min.
106. Reclaim the beads on an MPS for 1 min.

107. Conduct the following washes as described in Step 98.i–98.iv.
 - i. Wash the beads twice with 200 μ L of TWB.
 - ii. Wash the beads once with 100 μ L of 1 $\times$ BB.
 - iii. Wash the beads once with 100 μ L of 1 $\times$ NEBuffer 2.
 - iv. Wash the beads once with 25 μ L of 1 $\times$ NEBuffer 2.
108. Resuspend the beads in the amount of 1 $\times$ NEBuffer 2 equivalent to the starting volume of beads used in Step 97.

For example, if 10 μ L of Dynabeads MyOne Streptavidin C1 beads were used to pull down the library, then resuspend the library in 10 μ L of 1X NEBuffer 2.

Amplifying the Adapter-Modified Hi-C Library Using Paired-End PCR

109. Perform a PCR to titrate the number of cycles for library amplification.
 - i. Combine the following reagents in a 0.2-mL PCR tube.

Hi-C-coated streptavidin beads	3 μ L
PfuUltra II buffer (10 $\times$)	5 μ L
dNTPs (100 mM; 25 mM each)	0.4 μ L
PE1.0 primer (25 μ M)	0.7 μ L
PE2.0 primer (25 μ M)	0.7 μ L
PfuUltra II	1 μ L
H ₂ O (sterile)	39.2 μ L
 - ii. Amplify the library using the following program on a thermal cycler: 30 sec at 98°C; 6 cycles of 10 sec at 98°C, 30 sec at 65°C, and 30 sec at 72°C; and 2 min at 72°C.
 - iii. Vortex the PCR reaction and remove 2.0 μ L for gel electrophoresis.
 - iv. Quickly centrifuge the reaction and repeat Steps 109.ii and 109.iii, except run the PCR program for only 3 cycles.
 - v. Repeat Step 109.iv until a total of 18 cycles has been completed.
110. Perform gel electrophoresis of all reactions on a short 2% agarose gel in 0.5 $\times$ TBE (Fig. 1D). Quantify the amount of DNA product for each cycle number by comparison to a known concentration standard.
111. Choose a number of cycles for amplification that will yield at least 100 fmol of final Hi-C library when all of the beads are amplified, but that does not produce higher molecular mass artifacts which arise from overcycling. Choose the least number of cycles possible.

All libraries in a given experiment should be amplified using the same number of cycles.
112. Using the reaction setup described in Step 109.i, prepare enough reactions to amplify all of the remaining beads (3 μ L of beads/reaction).
113. Amplify the reactions using the thermal cycler program described in Step 109.ii with the optimal number of cycles determined in Step 111.
114. After amplification, pool all reactions together in a 1.7-mL tube. Add 1.8 volumes of AmPure XP beads to the pooled reactions.
115. Place the tube on an MPS for 1 min to collect the AmPure XP beads to the side of the tube.
116. While still on the MPS, remove the supernatant and discard.
117. While still on the MPS, wash the AmPure XP beads twice with 1 mL of 70% ethanol per wash as described in Steps 90–91. Air-dry the beads on the magnet.
118. Elute the DNA by resuspending the beads in 14 μ L of TLE buffer.

119. Place the tube on an MPS for 1 min to collect the beads to the side of the tube.
120. While still on the MPS, transfer the supernatant to a fresh 1.7-mL tube.
121. Quantify the Hi-C library DNA using the method of choice.

TROUBLESHOOTING

Problem (Step 68): The Hi-C efficiency is low.

Solution: This can be caused by a poor biotinylation reaction or a poor ligation reaction, or both. Repeat these reactions as needed.

RELATED INFORMATION

Hi-C provides the most comprehensive genome-wide analysis of any of the 3C-based techniques for studying chromatin conformation (Lieberman-Aiden et al. 2009; Belton et al. 2012). Classic 3C (Dekker et al. 2002) is described in Protocol: **Chromosome Conformation Capture (3C) in Budding Yeast** (Belton and Dekker 2015).

RECIPES

Binding Buffer (BB)

Reagent	Amount to add (for 100 mL)	Final concentration (1×)
Tris-HCl (1 M, pH 8.0)	500 μ L	5.0 mM
EDTA (500 mM)	100 μ L	0.5 mM
NaCl (5 M)	20 mL	1 M

For 2× BB, double the volume of each reagent added. Prepare in deionized H₂O. Store for up to 1 yr at room temperature.

Ligation Buffer for 3C (10×)

Reagent	Amount to add (for 1 L)	Final concentration (10×)
Tris-HCl (1 M, pH 7.5)	500 mL	500 mM
MgCl ₂ (1 M)	100 mL	100 mM
Dithiothreitol (DTT) (1 M)	100 mL	100 mM

Prepare in deionized H₂O. Store at −20°C in 15-mL aliquots.

Phenol:Chloroform (1:1)

In a chemical fume hood, adjust the pH of the phenol to 8.0 with Tris buffer. Shake vigorously. Mix 500 mL of phenol (pH 8.0) and 500 mL of chloroform in a 1-L glass bottle with a lid. Shake the mixture vigorously and let it separate overnight at 4°C. Store for up to 1 mo at 4°C.

Sodium Acetate (3 M, pH 5.2)

Dissolve 246.1 g of sodium acetate in 500 mL of deionized H₂O. Adjust the pH to 5.2 with glacial acetic acid. Allow the solution to cool overnight. Adjust the pH once more to 5.2 with glacial acetic acid. Adjust the final volume to 1 L with deionized H₂O and filter-sterilize.

TE Buffer

Reagent	Quantity (for 100 mL)	Final concentration
EDTA (0.5 M, pH 8.0)	0.2 mL	1 mM
Tris-Cl (1 M, pH 8.0)	1 mL	10 mM
H ₂ O	to 100 mL	

TLE Buffer

Reagent	Amount to add (for 1 L)	Final concentration
Tris-HCl (1 M, pH 8.0)	10 mL	10 mM
EDTA (0.5 M, pH 8.0)	200 μ L	0.1 mM

Prepare in deionized H₂O. Store for up to 1 yr at room temperature.

Tween Wash Buffer (TWB)

Reagent	Amount to add (for 100 mL)	Final concentration
Tris-HCl (1 M, pH 8.0)	500 μ L	5.0 mM
EDTA (500 mM)	100 μ L	0.5 mM
NaCl (5 M)	20 mL	1 M
Tween 20 (100%)	50.0 μ L	0.05%

Prepare in deionized H₂O. Store for up to 1 yr at room temperature.

TBE Buffer

Prepare a 5 $\times$ stock solution in 1 L of H₂O:

- 54 g of Tris base
- 27.5 g of boric acid
- 20 mL of 0.5 M EDTA (pH 8.0)

The 0.5 $\times$ working solution is 45 mM Tris-borate/1 mM EDTA. TBE is usually made and stored as a 5 $\times$ or 10 $\times$ stock solution. The pH of the concentrated stock buffer should be $\sim$ 8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10 $\times$ as opposed to 5 $\times$). However, 5 $\times$ stock solution is more stable because the solutes do not precipitate during storage. Passing the 5 $\times$ or 10 $\times$ buffer stocks through a 0.22- μ m filter can prevent or delay formation of precipitates.

REFERENCES

- Belton J-M, Dekker J. 2015. Chromosome conformation capture (3C) in budding yeast. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot085175.
- Belton J-M, McCord RP, Gibcus JH, Naumova N, Zhan Y, Dekker J. 2012. Hi-C: A comprehensive technique to capture the conformation of genomes. *Methods* 58: 268–276.
- Dekker J, Rippe K, Dekker M, Kleckner N. 2002. Capturing chromosome conformation. *Science* 295: 1306–1311.
- Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326: 289–293.

Cold Spring Harbor Protocols

Hi-C in Budding Yeast

Jon-Matthew Belton and Job Dekker

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot085209

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[Cell Biology, general](#) (1382 articles)
[Genome Analysis](#) (191 articles)
[Genomic DNA](#) (135 articles)
[Genomic Libraries](#) (66 articles)
[Libraries](#) (147 articles)
[Molecular Biology, general](#) (1293 articles)
[Yeast](#) (288 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>