NOV 19, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4mjgu4n

External link:
<https://doi.org/10.1038/s42003-021-02089-y>

Protocol Citation: Mitsutaka Kadota, Osamu Nishimura, Hisashi Miura, Kaori Tanaka, Ichiro Hiratani, Shigehiro Kuraku 2019. iconHi-C Protocol (ver. 1.0). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.4mjgu4n>

MANUSCRIPT CITATION:
 Multifaceted Hi-C benchmarking: what makes a difference in chromosome-scale genome scaffolding? Mitsutaka Kadota, Osamu Nishimura, Hisashi Miura, Kaori Tanaka, Ichiro Hiratani, Shigehiro Kuraku bioRxiv 659623; doi: <https://doi.org/10.1101/659623>

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iconHi-C Protocol (ver. 1.0)

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ABSTRACT

Hi-C, a derivative of chromosome conformation capture (3C) targeting the whole genome, was originally developed as a means for characterizing chromatin conformation and is recently employed widely in scaffolding nucleotide sequences obtained by *de novo* genome sequencing. To gain insights into the best practice of Hi-C scaffolding, we performed a multifaceted methodological comparison and optimized various factors during sample preparation, sequencing, and computation. As a result, we have identified some key factors for efficient Hi-C data acquisition, including preparation of tissues, library preparation conditions, and quality controls of prepared samples. By incorporating those optimal conditions revealed by our experience, we release this customized protocol designated the 'inexpensive and controllable Hi-C (iconHi-C) protocol'.

Protocol status: Working
We use this protocol and it's working

Created: Jun 23, 2019

Last Modified: Nov 19, 2019

PROTOCOL integer ID:
24971

Keywords: Hi-C, iconHi-C,
genome scaffolding,
chromosome

GUIDELINES

This protocol consists of the following sections:

[Section 1.](#) (DAY 0) Preparation of cells/tissue
[Section 2.](#) (DAY 0) Fixation of cells/tissue
[Section 3.](#) (DAY 0) Pre-determination of the amount of tissue to use for Hi-C
[Section 4.](#) (DAY 1) Restriction enzyme digestion
[Section 5.](#) (DAY 2) DNA fill-in and ligation
[Section 6.1.](#) (DAY 2) Hi-C DNA purification
[Section 6.2.](#) (DAY 3) Hi-C DNA purification
[Section 7.](#) (DAY 3) Hi-C DNA QC (QC1)
[Section 8.](#) (DAY 3) Removal of biotin from un-ligated DNA ends
[Section 9.](#) (DAY 3) Fragmentation and size selection of the Hi-C DNA
[Section 10.](#) (DAY 3) Enrichment of biotin-containing DNA
[Section 11.](#) (DAY 4) Hi-C library preparation
[Section 12.](#) (DAY 4) Quality control of the Hi-C library (QC2)

MATERIALS

Reagents and consumables

- 1.5 ml Protein LoBind tube (Eppendorf, cat. 0030108116)
Note: For cell/tissue samples.
- 1.5 ml DNA LoBind tube (Eppendorf, cat. 0030108051)
Note: For DNA samples.
- 2.0 ml Protein LoBind tube (Eppendorf, cat. 0030108132)
Note: For cell/tissue samples.
- 50 ml tube (Thermo Fisher Scientific, cat. 14-432-22)
Note: For cell/tissue samples.
- 0.2 ml PCR tube (INA OPTICA, cat. 3247-00)
- 384-well PCR plate (Applied Biosystems, cat. 4309849)
- Optical Adhesive Film (Applied Biosystems, cat. 4311971)
- Liquid nitrogen
- Mortar and pestle (AS ONE, cat. 2-9037-02)
- SK mill (Tokken, cat. SK-200)
- Stainless-steel tube (Tokken, cat. TK-AM5-SUS)
- Stainless-steel bullet (Tokken, cat. SK-100-DLC10)
- Tube holder (Tokken, cat. SK-100-TL)
- Douncer (Sigma-Aldrich, cat. D8938)
- PBS minus (Wako Pure Chemical, cat. 314-90185)
Note: Make a 1X solution with H₂O.
- 16% formaldehyde (Pierce, cat. 28906)
- Glycine (Wako Pure Chemical, cat. 077-00735)

Note: Make a 2.5 M solution with H₂O.

- 1 M Tris-HCl (pH 8.0) (Wako Pure Chemical, cat. 314-90065)
- 5 M NaCl (Nacalai Tesque, cat. 31334-51)
- 0.5 M EDTA (Invitrogen, cat. 15575-038)
- 10% SDS (Invitrogen, cat. 15553-035)
- Triton X-100 (Sigma-Aldrich, cat. T8787)

Note: Make a 20% solution (w/v) with H₂O.

- IGEPAL CA-630 (NP40) (Sigma-Aldrich, cat. 18896)

Note: Make a 10% solution (w/v) with H₂O.

- Tween 20 (Sigma-Aldrich, cat. P9416)

Note: Make a 10% solution (w/v) with H₂O.

- Proteinase inhibitor cocktail (Sigma-Aldrich, cat. P8340)
- Proteinase K solution (Nacalai Tesque, cat. 15679-64)
- RNase A (Takara, cat. U0505S)

Note: Make a 10 mg/ml solution with H₂O.

- Phenol/Chloroform/Isoamyl alcohol (25:24:1) (Wako Pure Chemical, cat. 311-90151)
- Glycogen solution (Thermo Fisher Scientific, cat. R0561)
- 2-Propanol (Nacalai Tesque, cat. 03065-35)
- Ethanol (Junsei, cat. 17065-1230)

Note: Make 70% solution (for EtOH precipitaton) and 80% solution (for AMPure purification) with H₂O.

- TE (pH 8.0) (Wako Pure Chemical, cat. 314-90021)
- EB (Qiagen, cat. 19086)
- Qubit dsDNA High Sensitivity Kit (Thermo Fisher Scientific, cat. Q32851)
- Agilent Bioanalyzer High Sensitivity DNA Kit (Agilent Technologies, cat. 5067-4626)
- Agilent TapeStation Genomic DNA ScreenTape (Agilent Technologies, cat. 5067-5365)
- Agilent TapeStation Genomic DNA Reagents (Agilent Technologies, cat. 5067-5366)
- Agilent TapeStation High Sensitivity D1000 ScreenTape (Agilent Technologies, cat. 5067-5584)
- Agilent TapeStation High Sensitivity D1000 Reagents (Agilent Technologies, cat. 5067-5585)
- NEBuffer 2 (New England Biolabs, cat. B7002S)
- NEBuffer 2.1 (New England Biolabs, cat. B7202S)
- NEBuffer DpnII (New England Biolabs, cat. B0543)
- DpnII (New England Biolabs, cat. R0543M)
- HindIII (New England Biolabs, cat. R3104M)
- ClaI (Takara, cat. 1034A)
- NheI (New England Biolabs, cat. R0131S)
- BSA solution (New England Biolabs, cat. B9000S)

- dNTP set (Thermo Fisher Scientific, cat. LS10297018)
Note: Make 1 mM and 10 mM solution with H₂O.
- Biotin-14-dATP (Thermo Fisher Scientific, cat. 19524016)
- Biotin-14-dCTP (Thermo Fisher Scientific, cat. 19518018)
- Klenow DNA polymerase (New England Biolabs, cat. M0210L)
- T4 DNA ligase (New England Biolabs, cat. M0202M)
- T4 DNA polymerase (New England Biolabs, cat. M0203S)
- Covaris microTUBE (Covaris, cat. 520045)
- Agencourt AMPure XP beads (Beckman coulter, cat. A63880)
- Magnet stand for the microtube (Thermo Fisher Scientific, cat. 12321D)
- Magnet stand for a PCR tube (Nippon Genetics, cat. FG-SSMAG2)
- Streptavidin beads (Thermo Fisher Scientific, cat. 11205D)
- KAPA LTP Library Preparation Kit (KAPA Biosystems, cat. KK8230)
- KAPA HiFi HotStart Ready Mix (KAPA Biosystems, cat. KK2600)
- KAPA HiFi HotStart Real-time PCR Master Mix (KAPA Biosystems, cat. KK2701)
- Illumina TruSeq compatible UDI adapter (PerkinElmer, cat. NOVA-514180)
- TPC mix (10 μM each)
Note: Mix two oligos; 5'-AATGATACGGCGACCACCGAG-3' and 5'-CAAGCAGAAGACGGCATACGAG-3'.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

Section 1. (DAY 0) Preparation of cells/tissue

- 1 Follow sample preparation procedure that matches the sample type; dissociated cells (e.g., cultured cells and nucleated red blood cells), fibrous tissue (e.g. muscle, kidney, intestine, heart), or soft tissue (e.g., liver, brain, testis, and embryonic tissues).

Step 1 includes a Step case.

Dissociated cells

Animal tissue

Alternative procedure for a soft tissue

step case

Dissociated cells

Sample preparation procedure for dissociated cells (e.g., cultured cells and nucleated red blood cells)

- 2 Dissociate cells, count cell number, and collect 1×10^7 cells in a microtube (1.5 or 2.0 ml).

Centrifuge the cells.

3

Centrifugation speed: 500 x g

Centrifugation time: 00:05:00

Centrifugation temperature: 4 °C

4

Remove the supernatant.

5

Put the tube on ice, and proceed immediately to fixation (Step 6).

Section 2. (DAY 0) Fixation of cells

6

Prepare the **fixing solution** and put on ice.

Reagent	Amount	Final Concentration
16% formaldehyde solution	1 ml	(1%)
PBS (-)	15 ml	
Total	16 ml	

Fixing solution

Note

Make fresh every time.

7

Take the sample tube out from the ice bucket (from Step 5) and add 1 mL - 1.5 mL ice-cold fixing solution .

- 8 Vortex until the cells are fully resuspended in fixing solution.
- 9 Incubate the cell-suspension for 00:10:00 in a heat block or a water bath set at 25 °C .
- 10 Quick spin the sample tube.
- 11 Add 1/20 volume of a 2.5 Molarity (M) glycine solution to quench the formaldehyde.
- 12 Vortex mix.
- 13 Put the sample tube on ice for ~ 00:01:00 .
- 14 Centrifuge the sample tube.

Centrifugation speed: 1000 x g
Centrifugation time: 00:05:00
Centrifugation temperature: 4 °C
- 15 Remove the supernatant.

16 Add 1 mL ice-cold PBS (-) . (1/2)

17 Vortex mix. (1/2)

18 Centrifuge the sample tube. (1/2)

Centrifugation speed: 1000 x g

Centrifugation time: 00:05:00

Centrifugation temperature: 4 °C

19 Remove the supernatant. (1/2)

20 Repeat the washing cycle (Steps 16-19) once more.

go to step #16

21 Resuspend the pellet in 1 mL 1.5 mL ice-cold PBS and aliquot the cell-suspension into microtubes (1.5 ml) at $1-2 \times 10^6$ cells/tube.

22 Centrifuge the sample tubes.

Centrifugation speed: 1000 x g

Centrifugation time: 00:05:00

Centrifugation temperature: 4 °C

23 Remove the supernatant.

- 24 Store the sample tubes in an ultra-low temperature freezer (e.g., -80 °C) until use.

Section 3. (DAY 0) Pre-determination of the amount of tissu.

25

Note

The pre-determination section quantitates the amount of DNA contained in a cell/tissue pellet prepared in the previous section. Pre-determination should always be performed for tissue samples, but it is optional when the DNA content in a pellet is estimable,. e.g., for cultured cells and nucleated red blood cells.

Section 4. (DAY 1) Restriction enzyme digestion

26

Note

This section includes the following steps.

1. Cell/tissue permeabilization - Steps 26 - 51
2. Restriction enzyme digestion - Steps 52 - 54

Note

The number of cells to use for Hi-C is determined based on the amount of DNA.

Use 1×10^6 cells for an animal with the genome size of 3-3.5 Gb (e.g., human and mouse), or 2×10^6 cells for an animal with the genome size of 1-1.5 Gb (e.g., chicken and western clawed frog).

For tissue samples, use a pellet that contains 2-10 μg of DNA.

Note

Follow cells/tissue resuspension steps as written, i.e., vortex mix or pipet mix.

Prepare the **permeabilization buffer 1 (PB1)**.

Reagent	Amount	Final Concentration
1 M Tris-HCl (pH 8.0)	400 µl	(10 mM)
5 M NaCl	80 µl	(10 mM)
10% (w/v) NP-40	800 µl	(0.2%)
H ₂ O	38.72 ml	
Total	40 ml	

Permeabilization buffer 1 (PB1)

Note

Filtrate and store at 4 °C .

- 27 Take (400 µL ×n) + 5% extra volume of PB1 in a new tube, add 1/100 vol of a proteinase inhibitor cocktail (PI), and put on ice.

Note

Add PI to PB1 just before use.

- 28 Take the frozen cells out from the freezer (from Step 24) and immediately add 400 µL PB1 (with PI) .

- 29 Vortex mix.

30 Incubate the sample tube on ice for 00:20:00 with periodical mixing every 00:05:00 - 00:10:00 .

31 Centrifuge the sample tube.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

32 Remove 300 µL of the supernatant using a P-1000 pipet.

33 Centrifuge the sample tube again.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

34 Remove the remaining supernatant using a P-100 or a P-200 pipet, and put the sample tube on ice.

35 .
Prepare the **permeabilization buffer 2 (PB2)** at Room temperature .

Reagent	Amount (x1 reaction)	Final Concentration
10X NEBuffer 2.1	25 µl	(1X)
10% (w/v) SDS	7.5 µl	(0.3%)
PI (100X)	2.5 µl	(1X)
H2O	215 µl	

Total	250 μ l	
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Permeabilization buffer 2 (PB2)

Note

Make fresh every time.
Make 5% extra volume.

35.1 Take 215 μ L H₂O in a tube of appropriate size.

35.2 Add 25 μ L 10X NEBuffer 2.1 .

35.3 Add 7.5 μ L 10% (w/v) SDS .

35.4 Add 2.5 μ L PI (100X) .

36 Add 250 μ L PB2 to the sample tube and pipet mix.

37 Incubate/shake the sample tube in a thermal mixer for 00:10:00 at 37 °C ,
950 rpm .

38 Add 28 µL 20% TritonX-100 to the sample tube.

39 Incubate/shake the sample tube in a thermal mixer for 00:10:00 at 37 °C ,
950 rpm .

40 Prepare the **DNA-preparation buffer** .

Reagent	Amount	Final Concentration
5 M NaCl	250 µl	(250 mM)
0.5 M EDTA	50 µl	(5 mM)
10% (w/v) SDS	500 µl	(1%)
H2O	4.2 ml	
Total	5 ml	

DNA-preparation buffer

Note

Store DNA-preparation buffer at Room temperature .

Warm the DNA-preparation buffer at 37 °C to dissolve precipitates formed during storage.

41 Take 8 µL (3%) aliquot (from Step 39) in a new 1.5 ml microtube as a pre-digest DNA control (ctr-1), add 42 µL DNA-preparation buffer and store at -20 °C until the end of DAY2.

42 Centrifuge the sample tube.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

43 Remove 200 µL of the supernatant using a P-200 pipet.

44 Centrifuge the sample tube again.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

45 Remove the remaining supernatant using a P-100 or a P-200 pipet, and put the sample tube on ice.

46 Prepare **washing buffer (WB)** with NEBuffer DpnII (for the DpnII digested sample).

Reagent	Amount (x1 wash cycle)	Final Concentration
10X NEBuffer DpnII	50 µl	(1X)
20 mg/ml BSA	2.5 µl	(0.1 mg/ml)
20% (w/v) TritonX-100	1.25 µl	(0.05%)
H2O	446.25 µl	
Total	500 µl	

Washing buffer (WB)

Note

Do not use NEBuffer 3.1 for the samples for DpnII digestion.

Make WB for HindIII digestion with NEBuffer 2.1 (that contains BSA).

Make 5% extra volume.

- 46.1 Take 446.25 µL H₂O in a tube of appropriate size.
- 46.2 Add 50 µL 10X NEBuffer DpnII .
- 46.3 Add 2.5 µL 20 mg/ml BSA .
- 46.4 Add 1.25 µL 20% (w/v) TritonX-100 .
- 47 Add 500 µL WB to the sample tube and vortex mix.
- 48 Centrifuge the sample tube.
- Centrifugation speed: 2000 x g
- Centrifugation time: 00:03:00 - 00:05:00
- Centrifugation temperature: 4 °C
- 49 Remove 400 µL of the supernatant using a P-1000 pipet.
- 50 Centrifuge the sample tube again.
- Centrifugation speed: 2000 x g
- Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

51 Remove the remaining supernatant using a P-100 pipet or a P-200 pipet, and put the sample tube on ice.

52 Prepare the **restriction-enzyme mix** (for DpnII digestion) on ice.

Reagent	Amount (x1 reaction)	Final Concentration
10X NEBuffer DpnII	20 µl	(1X)
20 mg/ml BSA	1 µl	(0.1 mg/ml)
DpnII (50 U/µl)	8 µl	(400 U)
20% Triton X-100	0.5 µl	(0.05%)
H2O	170.5 µl	
Total	200 µl	

Restriction-enzyme mix

Note

Do not use NEBuffer 3.1 for DpnII because non-specific cleavage (star activity) may be induced.

Use NEBuffer 2.1 (that contains BSA) for HindIII digestion.

For restriction enzymes at different concentration, e.g., HindIII at 100U/µl, adjust the volume with H2O.

Make 5% extra volume.

52.1 Take 170.5 µL H2O in a tube of appropriate size.

52.2 Add 20 µL 10X NEBuffer DpnII .

52.3 Add 1 μL 20 mg/ml BSA .

52.4 Add 8 μL DpnII (50 U/ μL) .

52.5 Add 0.5 μL 20% Triton X-100 .

53 Add 200 μL restriction-enzyme mix to the sample tube and pipet mix.

54 Incubate the sample tube in a thermal mixer for ~ 16:00:00 at 37 °C , 1100 rpm .

55 Take 6 μL (3%) aliquot in a new 1.5 ml microtube as a digested-DNA control (ctr-2), add 44 μL DNA-preparation buffer and store at -20 °C until the end of DAY 2.

Section 5. (DAY 2) DNA fill-in and ligation

56

Note

This section includes the following steps.

1. DNA fill-in reaction - Steps 56 - 76
2. Ligation reaction - Steps 77 - 79

Note

Follow cells/tissue resuspension steps as written, i.e., vortex mix or pipet mix.

Centrifuge the sample tube.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

57 Remove 150 µL of the supernatant using a P-200 pipet.

58 Centrifuge the sample tube again.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

59 Remove the remaining supernatant using a P-200 pipet or a P-100 pipet, and put the sample tube on ice.

60 Prepare the **washing buffer (WB)** with NEBuffer 2.1.

Reagent	Amount (x1 wash cycle)	Concentration
10X NEBuffer 2.1	50 µl	(1X)
20% (w/v) TritonX-100	1.25 µl	(0.05%)
H2O	448.75 µl	
Total	500 µl	

Washing Buffer (WB)

Note

Prepare buffer to perform total of four or two wash cycles (at Steps 61, 66 and 73), for the DpnII-digested sample or the HindIII-digested sample respectively.

60.1 Take 448.75 µL H₂O in a tube of appropriate size.

60.2 Add 50 µL 10X NEBuffer 2.1 .

60.3 Add 1.25 µL 20% (w/v) TritonX-100 .

61 Add 500 µL WB to the sample tube and vortex mix.

62 Centrifuge the sample tube.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

63 Remove 400 µL of the supernatant using a P-1000 pipet.

64 Centrifuge the sample tube again.

Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

65 Remove the remaining supernatant using a P-100 pipet or a P-200 pipet.

66 Repeat the washing cycle (Steps 61 - 65) twice more for the DpnII-digested sample (total of three wash cycles).

➡ go to step #61

Note

Additional washing cycle is not required for the HindIII-digested sample.

67 Put the sample tube on ice.

68 Prepare the **DNA fill-in mix** on ice.

Reagent	Amount (x1 reaction)	Final Concentration
10X NEBuffer 2	10 µl	(1X)
0.4 mM biotin-14-dATP	3.75 µl	(15 µM)
1 mM dCTP	1.5 µl	(15 µM)
1 mM dGTP	1.5 µl	(15 µM)
1 mM dTTP	1.5 µl	(15 µM)
Klenow DNA polymerase (5 U/µl)	6 µl	(30 U)
20% TritonX-100	0.25 µl	(0.05%)
H2O	75.5 µl	
Total	100 µl	

DNA fill-in mix for the DpnII-digested sample

Reagent	Amount	Final Concentration
10X NEBuffer 2	10 µl	(1X)
1 mM dATP	1.5 µl	(15 µM)
0.4 mM biotin-14-dCTP	3.75 µl	(15 µM)
1 mM dGTP	1.5 µl	(15 µM)
1 mM dTTP	1.5 µl	(15 µM)
Klenow DNA polymerase (5 U/µl)	3 µl	(15 U)
20% TritonX-100	0.25 µl	(0.05%)
H2O	78.5 µl	
Total	100 µl	

DNA fill-in mix for the HindIII-digested sample

Note

Make a DNA fill-in mix that contains either biotin-14-dATP or biotin-14-dCTP, for the DpnII-digested sample or the HindIII-digested sample respectively.
Make 5% extra volume.

68.1 Take 75.5 µL H₂O in a tube of appropriate size.

Note

Take 78.5 µL H₂O for the **DNA fill-in mix** for HindIII-digested sample.

68.2 Add 10 µL 10X NEBuffer 2 .

68.3 Add 3.75 μ L 0.4 mM biotin-14-dATP .

Note

Add 1.25 μ L 1 mM dATP for the **DNA fill-in mix** for HindIII-digested sample.

68.4 Add 1.25 μ L 1 mM dCTP .

Note

Add 3.75 μ L 0.4 mM biotin-14-dCTP for the **DNA fill-in mix** for HindIII-digested sample.

68.5 Add 1.25 μ L 1 mM dGTP .

68.6 Add 1.25 μ L 1 mM dTTP .

68.7 Add 6 μ L Klenow DNA polymerase (5 U/ μ l) .

Note

Add 3 μ L Klenow DNA polymerase (5 U/ μ l) for the **DNA fill-in mix** for HindIII-digested sample.

68.8 Add 0.25 μ L 20% TritonX-100 .

- 69 Add 100 μ L DNA fill-in mix to the sample tube and pipet mix.
- 70 Incubate the sample tube in a thermal mixer for 00:20:00 at 25 °C, 1100 rpm.
- 71 Centrifuge the sample tube.
- Centrifugation speed: 2000 x g
- Centrifugation time: 00:03:00 - 00:05:00
- Centrifugation temperature: 4 °C
- 72 Remove the supernatant using a P-100 pipet or a P-200 pipet.
- 73 Add 500 μ L WB to the sample tube and vortex mix.
- 74 Centrifuge the sample tube.
- Centrifugation speed: 2000 x g
- Centrifugation time: 00:03:00 - 00:05:00
- Centrifugation temperature: 4 °C
- 75 Remove 400 μ L of the supernatant using a P-1000 pipet.
- 76 Centrifuge the sample tube again.
- Centrifugation speed: 2000 x g

Centrifugation time: 00:03:00 - 00:05:00

Centrifugation temperature: 4 °C

77 Remove the remaining supernatant using a P-100 pipet or a P-200 pipet.

78 Put the sample tube on ice.

79 Prepare **ligation mix** on ice.

Reagent	Amount	Final Concentration
T4 DNA ligase buffer (10X)	10 µl	(1X)
T4 DNA ligase (2,000 U/ul)	2 µl	(4,000 CEU)
20% TritonX-100	0.25 µl	(0.05%)
H2O	87.75 µl	
Total	100 µl	

Ligation mix

Note

Prepare T4 DNA ligase buffer in small aliquots and store them at -20 °C when the buffer is thawed for the first time.

Always use an aliquot that is not thawed and frozen repeatedly.

Use 2,000 CEU of T4 DNA ligase for the HindIII-digested sample.

Make 5% extra volume.

79.1 Take 87.75 µL H2O in a tube of appropriate size.

- 79.2 Add 10 μ L T4 DNA ligase buffer (10X) .
- 79.3 Add 0.25 μ L 20% TritonX-100 .
- 79.4 Add 2 μ L T4 DNA ligase (2,000 U/ μ l) .
- 80 Add 100 μ L ligation mix to the sample tube and pipet mix.
- 81 Incubate the sample tube in a thermal mixer for 04:00:00 06:00:00 at 16 °C ,
1100 rpm .

Section 6.1. (DAY 2) Hi-C DNA purification

- 82 Prepare **DNA-extraction mix**.

Reagent	Amount (x1 reaction)	Final Concentration
1M Tris-HCl (pH 8.0)	2 μ l	(10 mM)
0.5M EDTA	2 μ l	(5 mM)
10% SDS	30 μ l	(1.5%)
5M NaCl	15 μ l	(375 mM)
20 mg/ml Proteinase K	10 μ l	(1 mg/ml)

H2O	141 μ l	
Total	200 μ l	

DNA-extraction mix

Note

Prepare DNA-extraction mix at Room temperature .

Make 5% extra volume.

82.1 Take 141 μ L H2O in a tube of appropriate size.

82.2 Add 2 μ L 1M Tris-HCl (pH 8.0) .

82.3 Add 2 μ L 0.5M EDTA .

82.4 Add 30 μ L 10% SDS .

82.5 Add 15 μ L 5M NaCl .

82.6 Add 10 μ L 20 mg/ml Proteinase K .

83 Add 200 µL DNA-extraction mix to the sample tube (from Step 81) and pipet mix.

Note

The total amount of the mixture will be 300 µL .

84 Add 200 µL DNA-extraction mix and 50 µL H₂O to the control tubes (ctr-1 from Step 41 and ctr-2 from Step 55) and pipet mix.

Note

The total amount of the mixture will be 300 µL .

85 Incubate in a thermal mixer for ~ 16:00:00 at 65 °C , 350 rpm .

Note

Use a thermal mixer equipped with a heated lid to avoid condensation of water on the lid. Alternatively, the incubation can be performed in a heated oven.

Section 6.2. (DAY 3) Hi-C DNA purification

86 Take the sample tube out from the thermal mixer (from Step 85) and let it cool down to Room temperature .

87 Add 5 µL RNase A (10 mg/ml) to the sample tube and mix gently.

88 Incubate sample tube in a thermal mixer for 00:20:00 at 37 °C , 800 rpm .

- 89 Add 5 μ L Proteinase K (20 mg/ml) to the sample tube and mix gently.
- 90 Incubate the sample tube in a thermal mixer for 02:00:00 at 55 °C, 800 rpm.
- 91 Take the sample tube out from the thermal mixer, let it cool down to Room temperature, and proceed with DNA extraction.
- 92 Add 300 μ L Phenol/Chloroform/Isoamyl alcohol solution to the sample tube and mix gently.
- 93 Centrifuge the sample tube.
- Centrifugation speed: 16000 x g
- Centrifugation time: 00:05:00
- Centrifugation temperature: Room temperature
- 94 Transfer ~ 250 μ L of the aqueous phase into a new 1.5 ml microtube.

- 95 Prepare **TE/NaCl solution**.

Reagent	Amount	Final concentration
1 M Tris-HCl (pH 8.0)	50 μ l	(10 mM)
5 M NaCl	250 μ l	(250 mM)
0.5 M EDTA (pH 8.0)	10 μ l	(1 mM)
H ₂ O	4.69 ml	
Total	5 ml	

TE/NaCl solution

Note

Store TE/NaCl solution at Room temperature .

96 Add 300 μL TE/NaCl solution to the sample tube containing Phenol and mix gently.

97 Centrifuge the sample tube.

Centrifugation speed: 16000 x g

Centrifugation time: 00:05:00

Centrifugation temperature: Room temperature

98 Transfer ~ 300 μL of the aqueous phase into the microtube containing the first aqueous phase.

Note

The total volume of the collected aqueous phase will be ~ 550 μL .

99 Add 1 μL glycogen solution (20 mg/ml) to the collected aqueous phase and mix gently.

100 Add 600 μL 2-propanol to the collected aqueous phase and mix gently until the solution becomes homogeneous.

101 Centrifuge the sample tube.

Centrifugation speed: 20000 x g

Centrifugation time: 00:30:00

Centrifugation temperature: 4 °C

102 Decant the supernatant, add 1 mL 70% EtOH, and mix gently to rinse the DNA pellet.

103 Centrifuge the sample tube.

Centrifugation speed: 20000 x g

Centrifugation time: 00:10:00

Centrifugation temperature: 4 °C

104 Decant the supernatant.

105 Centrifuge the sample tube again.

Centrifugation speed: 20000 x g

Centrifugation time: 00:05:00

Centrifugation temperature: 4 °C

106 Remove the supernatant completely with a P-100 or a P-200 pipet, and keep the lid open for ~ 00:01:00 to allow the ethanol to evaporate.

Note

Do not over dry the pellet.

107 Add 30 µL 50 µL EB and 10 µL EB to the Hi-C DNA and the control DNAs (ctr-1 and ctr-2) respectively.

Note

Mix gently to avoid shearing of the DNA.

Store DNA samples at 4 °C or -20 °C.

108 Quantitate DNA using 1 μL of the DNA sample with the Qubit dsDNA High Sensitivity Kit.

Section 7. (DAY 3) Hi-C DNA QC (QC1)

109 Take 1 μL - 2 μL of the DNA sample (ctr-1, ctr-2, and Hi-C DNA) from the previous section (Step 107) in a new PCR tube and add **EB** to adjust the concentration to 2-20 ng/ μL .

Note

The concentration of the DNA samples in a trio (ctr-1, ctr-2, and Hi-C DNA) should be within two-fold of difference, to accurately observe the size shift by Agilent Bioanalyzer or Agilent TapeStation.

110 Analyze 1 μL of the DpnII-digested DNA sample (ctr-2 and Hi-C DNA) using the Agilent Bioanalyzer with the DNA High Sensitivity chip, and 1 μL of the DpnII-digested DNA sample (ctr-1) using the Agilent TapeStation with the genomic tape.

Note

Only ctr-2 and Hi-C DNA of the DpnII digested samples are analyzed using the Agilent Bioanalyzer with the DNA High Sensitivity chip, because ctr-1 (pre-digested DNA), >50 kb in size, exceeds the limit of the DNA size that can be analyzed. Alternatively, the DpnII-digested DNA samples can be analyzed using the Agilent TapeStation with the genomic tape.

Note

For the HindIII-digested DNA samples, analyze 1 μL of ctr-1, ctr-2, and Hi-C DNA using Agilent TapeStation with the genomic tape.

Note

Only qualified Hi-C DNA showing the expected pattern of size shift, i.e., reduction in ctr-2 and recovery in Hi-C DNA, with no or minimum degree of DNA degradation in ctr-1, ctr-2 and Hi-C DNA, is used for the preparation of the Hi-C library.

- 111 Store Hi-C DNA at -20°C or proceed to the next section (Step 112).

Section 8. (DAY 3) Removal of biotin from un-ligated DNA e..

- 112 Take 250 ng - $2\text{ }\mu\text{g}$ of Hi-C DNA from the previous step (Step 111) in a 0.2 ml PCR tube and adjust the total volume to $30\text{ }\mu\text{L}$ with H_2O .

- 113 Prepare **T4 DNA polymerase mix** on ice.

Reagent	Amount	Final Concentration
10X NEBuffer 2.1	$5\text{ }\mu\text{l}$	(1X)
10 mM GTP	$0.5\text{ }\mu\text{l}$	(100 μM)
T4 DNA pol (3 U/ μl)	$1.67\text{ }\mu\text{l}$	(5 U)
H2O	$12.83\text{ }\mu\text{l}$	
Total	$20\text{ }\mu\text{l}$	

T4-DNA-polymerase mix for the DpnII-digested sample

Reagent	Amount	Final Concentration
10X NEBuffer 2.1	$5\text{ }\mu\text{l}$	(1X)
10 mM dATP	$0.5\text{ }\mu\text{l}$	(100 μM)

10 mM dGTP	0.5 µl	(100 µM)
T4 DNA pol (3 U/µl)	0.84 µl	(2.5 U)
H2O	13.16 µl	
Total	20 µl	

T4-DNA-polymerase mix for the HindIII-digested sample

Note

Make 5% extra volume.

113.1 Take 12.83 µL H2O in a tube of appropriate size.

Note

Take 13.16 µL H2O for the **T4-DNA-polymerase mix** for the HindIII-digested sample.

113.2 Add 5 µL 10X NEBuffer 2.1 .

113.3 Add 0.5 µL 10 mM dGTP .

Note

Add 0.5 µL 10 mM dATP and 0.5 µL 10 mM dGTP for the **T4-DNA-polymerase mix** for the HindIII-digested sample.

113.4 Add 1.67 µL T4 DNA pol (3 U/µl) .

Note

Add 0.84 µL T4 DNA pol (3U/µl) for the **T4-DNA-polymerase mix** for the HindIII-digested sample.

114 Add 20 µL T4-DNA-polymerase mix to the Hi-C DNA and mix gently.

115 Incubate the sample in a PCR machine for 00:30:00 at 37 °C , and 00:15:00 at 75 °C .

Section 9. (DAY 3) Fragmentation and size selection of the ..

116 Turn on the Covaris (S220 or E220) and the chiller.

117 Transfer the entire reaction from the previous section (Step 115) into a Covaris microTUBE and add 80 µL TE buffer .

Note

The total volume will be 130 µL .

118 Perform sonication

Duty factor: 5%

Peak incident power: 175

Cycles per burst: 200

Time: 00:01:00 ×2

Bath temperature: 7 °C

119 Transfer 120 µL of the fragmented DNA into a 1.5 ml microtube.

120 Add 72 µL (×0.6 amount) of AMPure XP beads to the fragmented DNA, vortex and incubate 00:05:00 at Room temperature .

Note

DNA larger than 600 bp are removed at Steps 120-122.

121 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

122 Transfer the supernatant into a new 1.5 ml microtube.

Note

The supernatant contains DNA smaller than 600 bp.

123 Add 108 µL (×0.9 amount) of AMPure XP beads to the supernatant, vortex mix, and wait 00:05:00 at Room temperature .

Note

DNA larger than 150 bp are collected.

124 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

- 125 Remove the supernatant using a P-200 pipet while the sample tube is still on the magnet.
- 126 Add 200 µL 80% EtOH to the beads while the sample tube is still on the magnet and wait 00:00:30 . (1/2)
- 127 Remove EtOH with P-200 pipet while the sample tube is still on the magnet. (1/2)
- 128 Repeat the washing cycle (Steps 126-127) once more.
go to step #126
- 129 Quick spin the sample tube, put on magnet and remove the residual EtOH completely using a P-10 or a P-20 pipet.
- 130 Air dry the beads for ~ 00:01:00 at Room temperature with the lid kept open.
- 131 Add 60 µL EB to the beads, vortex mix and incubate 00:02:00 at Room temperature .
- 132 Quick spin the sample tube, put on magnet and collect the eluate in a new PCR tube.

Section 10. (DAY 3) Enrichment of biotin-containing DNA

133 Prepare the 2X binding-and-washing buffer (BWB).

Reagent	Amount	Final Concentration
1 M Tris-HCl (pH 7.5)	400 μ l	(10 mM)
0.5 M EDTA (pH 8.0)	80 μ l	(1 mM)
5 M NaCl	16 ml	(2 M)
10% Tween20	80 μ l	(0.02%)
H ₂ O	23.44 ml	
Total	40 ml	

2X binding-and-washing buffer (BWB)

Note

Store at Room temperature .

133.1 Take 23.44 mL H₂O in a tube of appropriate size.

133.2 Add 400 μ L 1 M Tris-HCl (pH 7.5) .

133.3 Add 80 μ L 0.5 M EDTA (pH 8.0) .

133.4 Add 16 mL 5 M NaCl .

133.5 Add 80 μ L 10% Tween20 .

134 Prepare 1X BWB by diluting the 2X BWB with H₂O.

Note

Store at Room temperature .

135 Mix the bottle of the streptavidin beads and transfer (25 μ L $\times$ n) + 5% extra volume of beads into a new 1.5 ml microtube.

136 Put the tube on the magnetic and wait until the supernatant becomes clear.

137 Remove the supernatant, take off the tube from the magnet, add 1 mL 1X BWB , and vortex mix.

138 Quick spin the tube, put on magnet and wait until the supernatant becomes clear.

139 Remove the supernatant, take off the tube from the magnet, add (60 μ L $\times$ n) + 5% extra volume of **2X BWB**, and pipet mix.

140 Add 60 µL beads (in 2X BWB) to the size-selected Hi-C DNA prepared in previous section (from Step 132) and vortex mix.

Note

The total volume will be 120 µL .

141 Incubate the sample tube in a thermal mixer for 00:15:00 at 20 °C with periodical mixing for 00:00:10 at 2000 rpm every 00:03:00 .

142 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

143 Remove the supernatant while the tube is still on the magnet.

144 Take off the sample tube from the magnet.

145 Add 100 µL 1X BWB to the sample tube and vortex mix. (1/4)

146 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear. (1/4)

147 Remove the supernatant while the tube is still on the magnet.(1/4)

- 148** Take off the sample tube from the magnet.(1/4)
- 149** Repeat the washing cycle (Steps 145-148) three more times.
go to step #145
- 150** Add 100 µL EB and vortex mix.
- 151** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.
- 152** Remove the supernatant while the tube is still on the magnet.
- 153** Add 100 µL EB with the tube kept on the magnet.
- 154** Remove the supernatant, take off the sample tube from the magnet, quick spin, and put the sample tube back on the magnet.
- 155** Remove the residual EB with a P-10 or a P-20 pipet.

- 156 Resuspend the beads in 50 µL EB and store the sample tube at 4 °C until the next day (DAY 4).

Section 11. (DAY 4) Hi-C library preparation

157

Note

This section consists of the following steps.

1. End repair - Steps 157 - 175
2. A-tailing - Steps 176 - 193
3. Adapter ligation - Steps 194 - 212
4. Pre-PCR (releasing DNA off the streptavidin beads) - Steps 213 - 226
5. PCR cycle pre-determination - Steps 227 - 231
6. Amplification of the library for Hi-C library QC (QC2) - Steps 232 - 234
7. Library amplification - Steps 235 - 249

Note

Library preparation is performed using the KAPA LTP DNA library kit but in a 1/5 reaction volume of the original protocol and with an additional step for PCR cycle pre-determination.

Prepare the **end-repair mix** on ice.

Reagent	Amount
10X KAPA End Repair Buffer	1.4 µl
KAPA End Repair Enzyme Mix	1.0 µl
H2O	11.6 µl
Total volume	14 µl

End-repair mix

Note

Make 5% extra volume.

157.1 Take 11.6 µL H₂O in a tube of appropriate size.

157.2 Add 1.4 µL 10X KAPA End Repair Buffer .

157.3 Add 1 µL KAPA End Repair Enzyme Mix .

158 Quick spin the sample tube prepared in the previous section (from Step 156), put on magnet and wait until the supernatant becomes clear.

159 Remove the supernatant, add 14 µL end-repair mix to the sample tube, pipet mix, and proceed immediately to the next step.

160 Incubate for 00:30:00 at 20 °C (followed by a hold at 4 °C) in a PCR machine.

161 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

- 162** Remove the supernatant while the tube is still on the magnet.
- 163** Take off the sample tube from the magnet.
- 164** Add 100 µL 1X BWB to the sample tube and vortex mix. (1/4)
- 165** Remove the supernatant while the tube is still on the magnet.(1/4)
- 166** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear. (1/4)
- 167** Take off the sample tube from the magnet.(1/4)
- 168** Repeat the washing cycle (Steps 164-167) three more times.
go to step #164
- 169** Add 100 µL EB and vortex mix.
- 170** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

- 171** Remove the supernatant while the tube is still on the magnet.
- 172** Add 100 µL EB with the tube kept on the magnet.
- 173** Remove the supernatant, take off the sample tube from the magnet, quick spin, and put the sample tube back on the magnet.
- 174** Remove the residual EB with a P-10 or a P-20 pipet.
- 175** Close the lid and put on ice.
- 176** Prepare the **A-tailing mix** on ice.

Reagent	Amount
10X KAPA A-Tailing Buffer	1.0 µl
KAPA A-Tailing Enzyme	0.6 µl
H2O	8.4 µl
Total volume	10 µl

A-tailing mix

Note

Make 5% extra volume.

176.1 Take 8.4 µL H₂O in a tube of appropriate size.

176.2 Add 1 µL 10X KAPA A-Tailing Buffer .

176.3 Add 0.6 µL KAPA A-Tailing Enzyme .

177 Add 10 µL A-tailing mix to the sample tube (from Step 175), pipet mix, and proceed immediately to the next step.

178 Incubate 00:30:00 at 30 °C (followed by a hold at 4 °C) in a PCR machine.

179 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

180 Remove the supernatant while the tube is still on the magnet.

- 181** Take off the sample tube from the magnet.
- 182** Add 100 µL 1X BWB to the sample tube and vortex mix. (1/4)
- 183** Remove the supernatant while the tube is still on the magnet.(1/4)
- 184** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear. (1/4)
- 185** Take off the sample tube from the magnet.(1/4)
- 186** Repeat the washing cycle (Steps 182-185) three more times.
go to step #182
- 187** Add 100 µL EB and vortex mix.
- 188** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.
- 189** Remove the supernatant while the tube is still on the magnet.

- 190 Add 100 µL EB with the tube kept on the magnet.
- 191 Remove the supernatant, take off the sample tube from the magnet, quick spin, and put the sample tube back on the magnet.
- 192 Remove the residual EB with a P-10 or a P-20 pipet.
- 193 Close the lid and put on ice.
- 194 Prepare the **ligation-buffer mix** (without the ligase enzyme) on ice.

Reagent	Amount
5X KAPA Ligation Buffer	2.0 µl
H2O	6.0 µl
Total volume	8 µl

Ligation-buffer mix

Note

Make 5% extra volume.

194.1 Take 6 µL H₂O in a tube of appropriate size.

194.2 Add 2 µL 5X KAPA Ligation Buffer .

195 Add 8 µL ligation-buffer mix and 1 µL 1 µM Illumina TruSeq compatible adapter to the sample tube (from Step 191) pipet mix, and put on ice.

Note

In the case of multiplexed sequencing, adaptors should be balanced for their nucleotide composition at each sequence position of the index sequence.

When sequencing a single library, it is better that the library is made using a mixed index adapter (e.g., 0.5 µL 1 µM Illumina TruSeq compatible adapter 1 +

0.5 µL 1 µM Illumina TruSeq compatible adapter 2) to balance the nucleotide composition of the index sequence.

We recommend using the UDI (unique dual-indexed) adapter to identify and remove index-hopped reads in the Illumina sequencing platform.

196 Add 1 µL KAPA T4 DNA ligase to the sample tube, pipet mix, and proceed immediately to the next step.

197 Incubate for 00:15:00 at 20 °C (followed by a hold at 4 °C) in a PCR machine.

198 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

199 Remove the supernatant while the tube is still on the magnet.

- 200** Take off the sample tube from the magnet.
- 201** Add 100 µL 1X BWB to the sample tube and vortex mix. (1/4)
- 202** Remove the supernatant while the tube is still on the magnet.(1/4)
- 203** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear. (1/4)
- 204** Take off the sample tube from the magnet.(1/4)
- 205** Repeat the washing cycle (Steps 201-204) three more times.
go to step #201
- 206** Add 100 µL EB and vortex mix.
- 207** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

- 208 Remove the supernatant while the tube is still on the magnet.
- 209 Add 100 µL EB with the tube kept on the magnet.
- 210 Remove the supernatant, take off the sample tube from the magnet, quick spin, and put the sample tube back on the magnet.
- 211 Remove the residual EB with a P-10 or a P-20 pipet.
- 212 Close the lid and put on ice.
- 213 Prepare the **pre-PCR mix** on ice.

Reagent	Amount
2X KAPA HiFi Ready Mix	10 µl
10 µM TPC mix	0.9 µl
H2O	9.1 µl
Total volume	20 µl

Pre-PCR mix

Note

Make 5% extra volume.

213.1 Take 9.1 µL H₂O in a tube of appropriate size.

213.2 Add 10 µL 2X KAPA HiFi Ready Mix .

213.3 Add 0.9 µL 10 µM TPC mix .

214 Add 20 µL pre-PCR mix to the sample tube (from Step 212), pipet mix, and proceed immediately to the next step.

215 Perform 4 cycles of PCR amplification at 98°C 45 sec, 4 cycles of (98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min, and a hold at 4°C.

216 Put the sample tube on the magnetic and wait until the supernatant becomes clear.

217 Transfer the supernatant to a new PCR tube, add 20 µL (×1 volume) of AMPure XP beads, vortex mix, and wait 00:05:00 at Room temperature .

Note

DNA larger than 200 bp will bind to the AMPure XP beads.

- 218 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.
- 219 Remove the supernatant using a P-200 pipet while the sample tube is still on the magnet.
- 220 Add 200 µL 80% EtOH to the beads while the sample tube is still on the magnet and wait 00:00:30 . (1/2)
- 221 Remove EtOH with a P-200 pipet while the sample tube is still on the magnet. (1/2)
- 222 Repeat the washing cycle (Steps 220-221) once more.
go to step #220
- 223 Quick spin the sample tube, put on magnet and remove the residual EtOH completely using a P-10 or a P-20 pipet.
- 224 Air dry the beads for ~ 00:01:00 at Room temperature with the lid kept open.
- 225 Add 11 µL EB to the beads, vortex mix, and incubate 00:02:00 at Room temperature .

226 Quick spin the sample tube, put on magnet and collect the eluate in a new PCR tube.

227 Prepare the **real-time PCR mix** on ice

Reagent	Amount (x1 reaction)
2X KAPA HiFi HS real-time Mix	5 µl
10 µM TPC mix	0.35 µl
H2O	3.15 µl
Total volume	8.5 µl

Real-time PCR mix

Note

Make one extra reaction for the negative control that does not contain any DNA template.
Make 5% extra volume.

227.1 Take 3.15 µL H2O in a tube of appropriate size.

227.2 Add 5 µL 2X KAPA HiFi HS real-time Mix .

227.3 Add 0.35 µL 10 µM TPC mix .

- 228
- Dispense 8.5 µL real-time-PCR mix in a well of a PCR plate (384 well or 96 well) and add 1.5 µL of the pre-PCR product (from Step 224).
- 229
- Dispense 10 µL of each Fluorescence Standards (FS1~4) in the same plate in separate wells.
- 230
- Run real-time PCR in a ROX minus condition at, 98°C 45 sec, 20 cycles of (98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min.
- 231
- Determine the threshold-PCR-cycle (Ct) that reaches Fluorescence Standard 1 (FS1) but does not exceed Fluorescence Standard 2 (FS2).

Note

DNA for the library QC (QC2) is prepared by amplifying a small aliquot (1 µl) of the pre-PCR product with (Ct +3) cycles of PCR.
Hi-C library for sequencing is prepared by amplifying the remaining 8.5 µl of the pre-PCR product with the pre-determined Ct cycle.

- 232
- Prepare the **PCR-mix for Hi-C library QC (QC2)** on ice.

Reagent	Amount
2X KAPA HiFi HotStart Ready Mix	5 µl
10 µM TPC	0.45 µl
H2O	3.55 µl
Total volume	9 µl

PCR-mix for Hi-C library QC (QC2)

Note

Make 5% extra volume.

232.1 Take 3.55 µL H₂O in a tube of appropriate size.

232.2 Add 5 µL 2X KAPA HiFi HotStart Ready Mix .

232.3 Add 0.45 µL 10 µM TPC .

233 Dispense 9 µL of the **PCR-mix for Hi-C library QC (QC2)** in a PCR tube, and add 1 µL of the pre-PCR product (from Step 224).

234 Perform PCR amplification at, 98°C 45 sec, (Ct +3) cycles of (98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min, and a hold at 4°C.

Note

Proceed to Step 250 in the next section after the PCR.

235 Prepare the **PCR-mix for Hi-C library amplification**.

Reagent	Amount
2X KAPA HiFi HotStart Ready Mix	10 µl
10 µM TPC	0.9 µl
H ₂ O	0.6 µl

Total volume	11.5 μ l
--------------	--------------

PCR-mix for Hi-C library amplification

Note

Make 5% extra volume.

235.1 Take 0.6 μ L H₂O in a tube of appropriate size.

235.2 Add 10 μ L 2X KAPA HiFi HotStart Ready Mix .

235.3 Add 0.9 μ L 10 μ M TPC .

236 Add 11.5 μ L of the **PCR-mix for Hi-C library amplification** to the tube containing the pre-PCR product (from Step 226).

237 Perform PCR amplification at, 98°C 45 sec, (Ct) cycles of (98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min, and a hold at 4°C.

238 Add 20 μ L (×1 volume) of AMPure XP beads, vortex mix, and wait 00:05:00 at Room temperature .

- 239** Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.
- 240** Remove the supernatant using a P-200 pipet while the sample tube is still on the magnet.
- 241** Add 200 µL 80% EtOH to the beads while the sample tube is still on the magnet and wait 00:00:30 . (1/2)
- 242** Remove EtOH with a P-200 pipet while the sample tube is still on the magnet. (1/2)
- 243** Repeat the washing cycle (Steps 241-242) once more.
go to step #241
- 244** Quick spin the sample tube, put on magnet and remove the residual EtOH completely using a P-10 or a P-20 pipet.
- 245** Air dry the beads for ~ 00:01:00 at Room temperature with the lid kept open.
- 246** Add 30 µL EB to the beads, vortex mix, and incubate 00:02:00 at Room temperature .
- 247** Quick spin the sample tube, put on magnet and collect the eluate in a new microtube (1.5 ml).

248 Quantitate the DNA using 1 μL of the DNA sample with the Qubit dsDNA High Sensitivity Kit.

249 Analyze the size-distribution using 2 μL of the library DNA using the Agilent TapeStation with the High Sensitivity D1000 tape.

Section 12. (DAY 4) Quality control of the Hi-C library (QC2)

250 Add 10 μL ($\times 1$ volume) of AMPure XP beads to the PCR product from the previous section (Step 234), vortex mix, and wait 00:05:00 at Room temperature .

Note

DNA larger than 200 bp will bind to the AMPure XP beads.

251 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

252 Remove the supernatant using a P-200 pipet while the sample tube is still on the magnet.

253 Add 200 μL 80% EtOH to the beads while the sample tube is still on the magnet and wait 00:00:30 . (1/2)

254 Remove EtOH with a P-200 pipet while the sample tube is still on the magnet. (1/2)

- 255
- Repeat the washing cycle (Steps 253-254) once more.
- ➡ go to step #253
- 256
- Quick spin the sample tube, put on magnet and remove the residual EtOH completely using a P-10 or a P-20 pipet.
- 257
- Air dry the beads for ~⌚ 00:01:00 at 🌡 Room temperature with the lid kept open.
- 258
- Add 🧴 5 µL EB to the beads, vortex mix, and incubate ⌚ 00:02:00 at 🌡 Room temperature .
- 259
- Quick spin the sample tube, put on magnet and collect the eluate in a new PCR tube.
- 260
- Quantitate the DNA using 🧴 1 µL of the DNA sample with the Qubit dsDNA High Sensitivity Kit.
- 261

Prepare the **restriction-mix for QC2** with or without the restriction enzyme (RE)

Reagent	Amount
10X buffer	1 µl
RE (10 U/µl) or H2O	0.3 µl
20 mg/ml BSA	0.05 µl

H2O	μl
Total volume	μl

Restriction-mix for QC2

Note

Adjust the volume of H₂O based on the concentration of the amplified library DNA.

The restriction enzyme for the DpnII-digested library is ClaI.

The restriction enzyme for the HindIII-digested library is NheI.

Make 5% extra volume.

262 Dispense the **restriction-mix for QC2** (with or without the RE) to a PCR tube, add **10 ng** of the amplified library DNA (from Step 259), adjust the total volume of the reaction to **10 μL**, and incubate **00:30:00** at **37 °C**.

263 Add **18 μL** (×1.8 volume) of AMPure XP beads, vortex mix, and wait **00:05:00** at **Room temperature**.

Note

DNA larger than 100 bp will bind to the AMPure XP beads.

264 Quick spin the sample tube, put on magnet and wait until the supernatant becomes clear.

265 Remove the supernatant using a P-200 pipet while the sample tube is still on the magnet.

266 Add **200 μL 80% EtOH** to the beads while the sample tube is still on the magnet and wait **00:00:30**. (1/2)

- 267** Remove EtOH with a P-200 pipet while the sample tube is still on the magnet. (1/2)
- 268** Repeat the washing cycle (Steps 266-267) once more.
go to step #266
- 269** Quick spin the sample tube, put on magnet and remove the residual EtOH completely using a P-10 or a P-20 pipet.
- 270** Air dry the beads for ~ 00:01:00 at Room temperature with the lid kept open.
- 271** Add 10 µL EB to the beads, vortex mix, and incubate 00:02:00 at Room temperature .
- 272** Quick spin the sample tube, put on magnet and collect the eluate in a new PCR tube.
- 273** Analyze the size difference between the RE(-) and the RE(+) samples using 2 µL of DNA with the Agilent TapeStation using the High Sensitivity D1000 tape.

Note

Place RE(-) and RE(+) samples for the same library side-by-side for easy comparison.