

## Hi-C protocol for analysis of plant nuclear chromatin interactions

This protocol is reproduced and adapted from:

Liu C (2017) **In Situ Hi-C Library Preparation for Plants to Study Their Three-Dimensional Chromatin Interactions on a Genome-Wide Scale**. *Methods Mol Biol.* doi: 10.1007/978-1-4939-7125-1\_11

Wang N, Liu C (2020) **Study of Cell-Type-Specific Chromatin Organization: In Situ Hi-C Library Preparation for Low-Input Plant Materials**. *Methods Mol Biol.* doi: 10.1007/978-1-0716-0179-2\_9

Dong P, Zhong S (2020) **Characterization of Plant 3D Chromatin Architecture, In Situ Hi-C Library Preparation, and Data Analysis**. *Methods Mol Biol.* 2020;2093:147-157. doi: 10.1007/978-1-0716-0179-2\_11

### i. Summary

The nuclear space is a dynamic environment in which DNA molecules interact across time, space and scale. Within the nucleus different yet adjacent chromosomes are co-regulated through shared chromatin modifications or the influence of global enhancer or repressor units. The interactions between adjacent chromosomes can be analysed through Hi-C, a technique that takes a whole genome view on samples obtained by chromosome conformation capture. This technique has been used in many different plant species and this article provides a consensus protocol that is merged from those used in two expert labs.

### ii. Keywords

Chromosome Conformation Capture, Hi-C, Genome organisation

## 1. Introduction

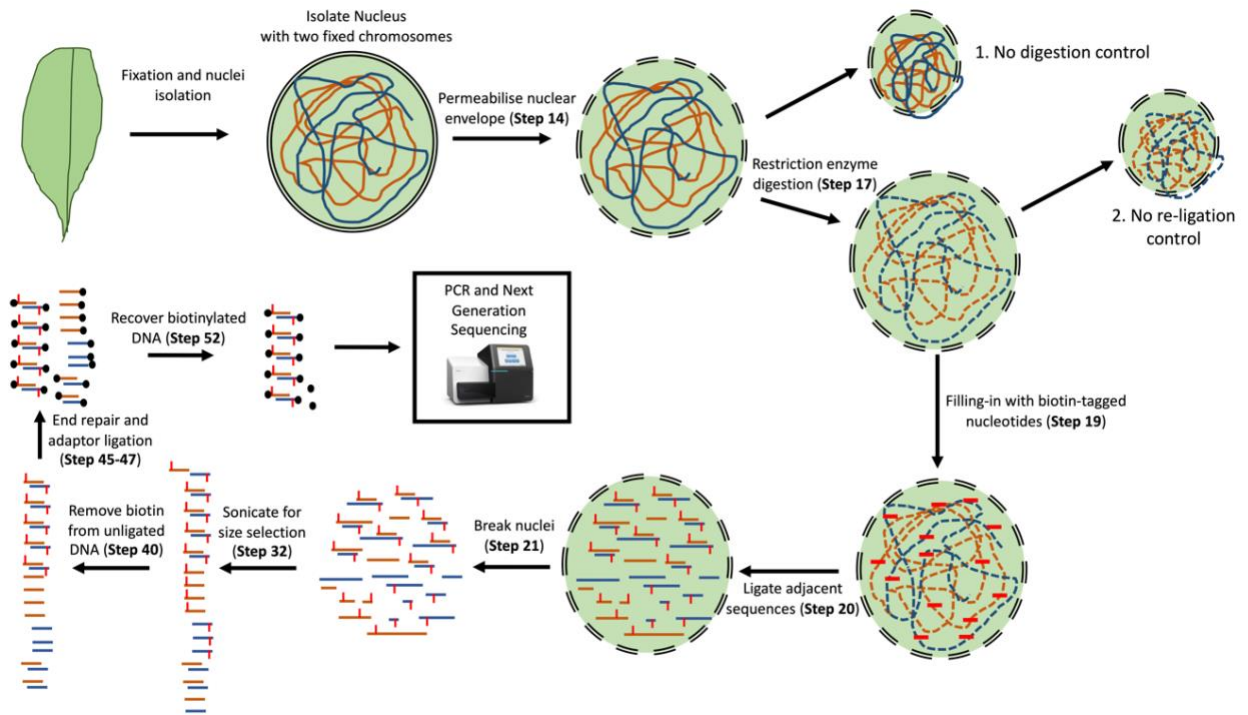
All eukaryotic genomes are complex across scales; from the variation in primary DNA sequence, changes in chromatin structure, their epigenetic modifications and higher-order intra-chromosome and inter-chromosome organisation. There is strong evidence that segments of different chromosomes that lie adjacent to each other within the nuclear space exhibit aspects of co-regulation (Grob *et al*, 2013; Concia *et al*, 2020). This might be through the shared epigenetic state of their chromatin or through the influence of enhancing or repressing elements on genome access and transcriptional control.

Chromosome conformation capture (3C) is a technique that allows the analysis of adjacent chromosomal regions and was first published in 2002 (Dekker *et al*, 2002). This technique involves fixing DNA in its native state within the nucleus, whole genome restriction digestion, filling-in of digested ends and subsequent re-ligation of associated regions into chimeric fragments, which can be analysed by simple PCR (**Figure 1**). Hi-C extends this technique to the whole genome scale through use of next generation sequencing following biotin-mediated capture and sequencing of chimeric fragments.

Recently this method has been used to analyse the genome organisation of a surprisingly high number of plant species including those of eggplant, sponge gourd or melon (Wei *et al*, 2020; Wu *et al*, 2020; Yang *et al*, 2020)! Although the main aspects of these procedures are invariant, as with most protocols there are slight lab-specific differences in methodologies.

The lab of Chang Liu from the Institute of Biology at the [University of Hohenheim](#) are experts in this research area and over the past year have used Hi-C for the analysis of chromosomal relationships in *Arabidopsis*, *Wheat* and *Marchantia* (Ariel *et al*, 2020; Concia *et al*, 2020; Karaaslan *et al*, 2020; Montgomery *et al*, 2020). Similarly, members of Silin Zhong's lab from the Chinese University of Hong Kong have recently used Hi-C to analyse genome structure in maize, foxtail millet, rice, tomato and sorghum (Dong *et al*, 2017; Dong *et al*, 2020).

Protocols from these two expert labs were published in recent editions of *Methods in Molecular Biology* (Liu C, 2017; Wang and Liu, 2020; Dong and Zhong., 2020). In this article we compare these methods and hope to present a combined consensus protocol that will help researchers new to this subject area.



**Figure 1: Overview of Hi-C protocol.**

## 2. Materials

### > TF buffer

- 10 mM potassium phosphate, pH 7.0
- 50 mM NaCl
- 0.1M sucrose.
- 1% Paraformaldehyde (v/v), made from paraformaldehyde stock

### > Paraformaldehyde stock (usually 37.5%).

### > Nuclei isolation buffer **[NOTE 1]:**

#### **Liu lab** (Wang and Liu, 2020):

- 20 mM HEPES (pH 8)
- 250 mM sucrose
- 1 mM MgCl<sub>2</sub>
- 5 mM KCl
- 40% (v/v) Glycerol
- 0.25% Triton X-100.

Add following components freshly before use to make it complete:

- 0.1 mM PMSF (final)
- 0.1% (v/v) 2-mercaptoethanol (final)

#### **Zhong lab** (Dong P, Zhong S, 2020):

- 0.4 M sucrose (in 1x TE buffer)
- 10 mM Tris-HCl (pH 7.5)
- 1 mM EDTA

- Freshly add protease inhibitor tablet (for example:  
<https://www.sigmaaldrich.com/catalog/product/roche/04693159001?>)  
10% Triton X-100 to a final concentration of 1% (v/v)  
0.1% (v/v) 2-mercaptoethanol (final)
- > DAPI solution:  
1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) dissolved in PBS (phosphate-buffered saline).
  - > Percoll gradient buffer (optional):  
0.25 M sucrose in 95% Percoll.  
  
To prepare a 50 mL Percoll gradient buffer  
4.2 g of Sucrose  
50 µL 1 M Tris-HCl (pH 7.5)  
Top up the volume with Percoll to 50 mL.
  - > SDS buffer:  
50 mM Tris, pH 8.0  
1% SDS (from 10% stock)  
10mM EDTA (from stock at 0.5M pH 8)
  - > Regular deoxynucleotide triphosphates (in separate tubes):  
10 mM dATP  
10 mM dGTP  
10 mM dTTP.
  - > Biotin-labeled dCTP: 0.4 mM biotin-14-dCTP (Thermo Fisher Scientific,  
<https://www.thermofisher.com/order/catalog/product/19518018?de&en#/19518018?de&en>)
  - > RNaseA. For example:  
<https://www.thermofisher.com/order/catalog/product/EN0531?de&en#/EN0531?de&en>
  - > AMPure XP beads: <https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/pcr>
  - > T4 Polymerase. For example from NEB: <https://international.neb.com/products/m0203-t4-dna-polymerase#Product%20Information>
  - > NEBNext Ultra II DNA library Preparation Kit: <https://international.neb.com/products/e7805-nebnext-ultra-ii-fs-dna-library-prep-kit-for-illumina#Product%20Information>
  - > Dynabeads® MyOne™ Streptavidin C1:  
<https://www.thermofisher.com/order/catalog/product/65001#/65001>
  - > TWB buffer:  
5mM Tris-HCl pH 8.0  
0.5 mM EDTA  
1M NaCl  
0.05% Tween
  - > Sonicated salmon sperm DNA. For example:  
<https://www.thermofisher.com/order/catalog/product/15632011?de&en#/15632011?de&en>

- > Binding buffer (BB):
  - 10 mM Tris-HCl pH 8.0
  - 1 mM EDTA
  - 2M NaCl

### 3. Methods

#### 3.1 Tissue Fixation

Both methods use vacuum filtration to fix samples in a phosphate-based formaldehyde solution, stopped by glycine solution, followed by water removal and flash freezing in liquid nitrogen.

1. Grow plants as needed due to specific protocol. Immerse up to 1g of tissue into TF buffer with 1% formaldehyde [**NOTE 2**]
2. Apply vacuum infiltration for 20minutes. Add glycine to final concentration of 0.125M to stop the fixation.
3. Mix well and apply vacuum infiltration for a further 15minutes.
4. Rinse samples in chilled water and blot tissues dry on tissue paper
5. Flash freeze samples on liquid nitrogen.
6. Proceed with nuclei isolation or store samples at -80C indefinitely

#### 3.2 Nuclei Isolation

Both methods grind samples under liquid nitrogen, resuspend in nuclear isolation buffer, filtered and centrifuged until a clean white pellet is obtained that lacks chloroplast contamination (green slurry in the pellet can be carefully discarded at each step).

The simple protocol below can be used for tissue samples that contain a low amount of starch, such as those obtained from Arabidopsis. However in tissues where starch contamination might be a major issue then use of a Percoll gradient can be used after **Step 9** to obtain a cleaner nuclear preparation.

7. Grind samples to a fine powder under liquid nitrogen and resuspend in chilled nuclei isolation buffer (10ml or 5 volumes of powder, whichever is the lesser amount) [**NOTE 3**].
8. Filter samples through a double-layered miracloth (or cell strainer with pore size 40  $\mu\text{m}$ ) and collect flow through in a 50ml Falcon tube.
9. Centrifuge at 3500 rcf for 10minutes at 4C and carefully discard supernatant [**NOTE 4**][**NOTE 5**].
10. Resuspend in 1ml nuclei isolation buffer and using a cut-off pipette tip transfer supernatant to a 0.2ml tube [**NOTE 6**].
11. Centrifuge at 3000 rcf for 10minutes and discard supernatant.

#### 3.3 Chromatin Digestion, DNA Ligation and Purification

12. Resuspend pellet in 300ul 1XNEB DpnII Buffer [**NOTE 7**]
13. Centrifuge at 3000g for 5 min at 4°C and discard the supernatant.
14. Gently resuspend the pellet in 150 $\mu\text{l}$  of 0.5% SDS
15. Incubate at 62 °C for 5 min, after which split the nuclei sample into 3 separate tubes of 50ul [**NOTE 8**]
16. To each tube, add 145 ul of water and 25 ul of 10% triton X-100, gently mix, and incubate at 37 °C for 15 min.
17. Add 25 ul of 10X NEB DpnII Buffer, 50U of DpnII and digest chromatin overnight at 37 °C (no shaking or rotating).

-----OVERNIGHT BREAK-----

18. Inactivate the DpnII by incubating the tubes at 62°C for 20 min.
19. Add buffer with 1 ul of 10 mM dTTP, 1ul of 10 mM dATP, 1ul of 10 mM dGTP, and 25 ul of 0.4 mM biotin-14-dCTP, 14 ul water. Then add 4 ul Klenow (40U) and incubate at 37 °C for at least 2 hr **[NOTE 9]**.
20. Add 663 ul water, 120ul 10x ligation buffer, 100 ul 10% Triton-100, 20 U (4000 units) T4 DNA ligase (master mix of 900 µl), mix gently and incubate at room temperature for 4 hr **[NOTE 10]**
21. Centrifuge at 1000 rcf for 3 min, discard supernatant, and harshly resuspend nuclei pellet with 750µl SDS buffer. As nuclei needs to be broken-up at this stage, a vortex can be used to resuspend this pellet.
22. Add 10µl Proteinase K and incubate at 55 °C for 30 min.
23. Add 30µl 5M NaCl, incubate at 65 °C overnight

-----OVERNIGHT BREAK-----

24. To remove contaminating RNA, add 1µL of RNaseA (10 mg/mL) and incubate at 37 °C for 30 min.
25. To purify the DNA, add equal volume of chloroform, vortex, and centrifuge at 10,000 g at 4 °C for 5 min.
26. Transfer the upper aqueous phase to a new tube, and add 1/10 volume of 3 M sodium acetate, 1µL of glycogen (10 mg/mL) and equal amount of isopropanol (v/v). Mix by inversion and incubate in -20 °C for 2 hours.
27. Spin down the DNA at maximum speed for 20 minutes at 4 °C. Wash the pellet with 80% ethanol, centrifuge for 5minutes at maximum speed at 4 °C.
28. Air dry the pellet in a heat block 37°C for 10 min (or until dry).
29. Resuspend in 100 ul 10 mM Tris pH 8.0.
30. Measure DNA concentration as accurately as possible (e.g., with Qubit Fluorometric Quantification). The total amount of DNA recovered is typically in the range of 2–20 µg. Recovered DNA can be stored long term at -20 °C.

### 3.4 DNA Manipulation and Size Selection

The ordering in this section varies between protocols. As outlined below the **Liu lab** first sonicate samples and then treat with T4 polymerase to remove biotin-labelled cytosine nucleotides from unligated DNA. In contrast the **Zhong lab** first remove the unligated biotinylated cytosines prior to sonication.

The Liu protocol reproduced below includes a double step of using AMPure XP beads. This should allow for isolation of a more purified sample yet if there are limitations in the amount of absolute DNA then the Zhong lab protocol of could be used (Dong and Zhong, 2020).

31. Begin with 3µg of DNA **[NOTE 11]**
32. Sonicate DNA to achieve desired fragment size range (250–500bp) **[NOTE 12]**
33. Recover DNA with any standard PCR purification kit that uses columns and elute DNA with 100 µl 10 mM Tris pH8 **[NOTE 13]**
34. Use AMPure XP beads to remove DNA molecules larger than 500bp **[NOTE 14]**. For 100 µl of DNA from **Step 33**, mix with 55 µl AMPure XP beads and incubate at RT for 10 min. **[NOTE 15]**
35. Reclaim the beads on a magnetic tube rack and transfer all supernatant to a new tube (**Keep Supernatant**).
36. Add a further 25 µl AMPure beads to the supernatant, mix, incubate at RT for 20 min **[NOTE 16]**
37. Reclaim beads (**discard supernatant**)
38. Wash beads with 80% EtOH (without disturbing them)

39. Dry beads, elute with DNA with 20µl 10 mM Tris pH8.0.

### 3.4 Remove Biotin from unligated cytosine nucleotides

40. To the 20µl of eluted DNA from **Step 39**, add 5µl of (10x) buffer for T4 DNA polymerase, 0.5µl of 10 mM dTTP, 0.5µl of 10 mM dATP, 5 U of T4 DNA polymerase, water to top up the volume to 50µl. Incubate at 20°C for 30 min.

**[NOTE 17]**

41. Stop the reaction by adding 2µl 0.5 M EDTA pH 8.0

42. Recover DNA with AMPure XP beads (v:v, 1:1), mix, incubate at RT for 20 min.

43. Reclaim beads, discard supernatant, wash beads with 80% EtOH (without disturbing them), Dry beads.

44. Elute with DNA with 20 µl 10 mM Tris pH8.0.

### 3.5 End repair and Adaptor ligation

This section of the protocol repairs damaged DNA ends using Klenow fragment and ligates the adaptors needed for next generation sequencing. The **Liu lab** protocol uses the NEBNext Ultra II DNA library prep kit, which allows for end repair and dA-tailing to be performed in the same tube, reducing the need for clean-up and associated sample loss **[NOTE 18]**.

45. Mix 20 µl of DNA (from **Step 44**) with 1.2 µl of Ultra II End Prep Enzyme Mix and 2.8 µl of Ultra II End Prep Reaction Buffer, incubate at 20°C for 30 min followed by incubation at 65°C for 30 min.

46. Sequentially add 12 µl of Ultra II Ligation Master Mix, 0.4 µl of Ligation Enhancer and 1 µl of Adaptor for Illumina sequencing. Mix well and incubate at 20 °C for 15 min.

47. Add 1.2 µl of USER Enzyme, mix well and incubate at 37 °C for 15 min.

48. Add 30 µl of AMPure XP beads, mix, incubate at RT for 10 min **[NOTE 19]**.

49. Reclaim beads on a magnetic rack, discard supernatant, wash beads with 80% EtOH (without disturbing them)

50. Dry beads, elute with DNA with 40 µl 10 mM Tris pH8.

### 3.6 Biotin affinity pulldown (~ 30 min)

In **Step 19** a biotin-labelled dC nucleotide was added to the reaction mix. This section uses a biotin-affinity pulldown to recover the DNA with the biotin incorporated nucleotides. The **Zhong lab** protocol introduces this step prior to end-repair and adaptor ligation so researchers may need to vary their protocol to understand which has best results in their hands. Both protocols advise using Dynabeads® MyOne™ Streptavidin C1 for recover biotin-labelled nucleotides.

51. Rinse 10 µl of magnetic streptavidin beads twice with 100 µl TWB buffer, during the second rinse, add 5 µg of sonicated salmon sperm DNA.

52. Resuspend the beads in 2x binding buffer (BB) with 0.1% Tween and add equal volume of Hi-C DNA obtained from **Step 50** (~40 µl).

53. Incubate at RT for 15 min with stirring, recover the beads on a magnetic rack and remove the supernatant

54. Resuspend beads with 1 ml 1x TWB buffer and transfer to a new tube.

55. Wash beads twice with 400 µl of 1x TWB buffer (**Steps 55-57** all on magnetic rack)

56. Wash beads with 200 µl 10 mM Tris-HCl pH 8.0.



57. Resuspend beads with 25  $\mu$ l 10 mM Tris-HCl pH 8.0.

### 3.6 Final PCR to amplify targets for next generation sequencing

58. From **Step 57** use 0.5 $\mu$ l streptavidin beads bound with Hi-C library as the template to run a trial PCR to determine the optimal PCR cycle **[NOTE 20]**
59. In a 10  $\mu$ l PCR reaction system use the following reaction mix: 0.5  $\mu$ l of beads, 5  $\mu$ l of Ultra II Q5 Master Mix, 0.5  $\mu$ l of Universal Primer, 0.5  $\mu$ l of selected Index Primer and 3.5  $\mu$ l of water.
60. At first use a PCR with up to 15 cycles and analyze samples with a DNA gel **[NOTE 21]**.
61. If test PCR is successful it will generate a smear between 400-700bp **[NOTE 22]**
62. According to results from test PCR (**Step 61**) set up a final PCR in a 20 $\mu$ l total volume with 5  $\mu$ l of beads.
63. Purify the product with AMPure XP beads for sequencing **[NOTE 23]**. Add 18 $\mu$ l of AMPure XP beads, mix, incubate at RT for 10 min.
64. Reclaim beads on a magnetic rack, discard supernatant, wash beads with 80% EtOH (without disturbing them)
65. Dry beads, elute DNA with 30 $\mu$ l 10mM Tris pH8
66. Quantify the Hi-C library with a Bioanalyzer
67. Samples will then enter the pipeline for analysis by next generation sequencing

#### 4. Experimental Notes:

**NOTE 1:** The composition of nuclei isolation buffer differs between the two labs, which is due to personal preference. This does not affect downstream processing of the samples.

**NOTE 2:** In Arabidopsis, authors used up to 1g of tissue. For tomato or other soft fruits up to 20g tissue can be used. This needs to be determined by the particular experiment.

**NOTE 3:** Tissue from larger samples will generate a higher amount of tissue so the amount of nuclei isolation buffer used will vary in an experiment-specific manner. The resuspended slurry should be easily pipetted through the filter.

**NOTE 4:** After centrifugation at low speed the pellet will not strongly adhere to the tube so care must be taken to not disturb the pellet during removal of supernatant. If needed take a cautious approach by adding extra buffer and recentrifuge the sample.

**NOTE 5:** As a reference point, the following Hi-C protocol works best if the pellet size after **Step 9** is comparable to a volume of 100 µl water. For larger pellets, a Percoll density gradient can be used for better separation:

- Resuspend the nuclei pellet with at least 10 volumes of chilled Percoll gradient buffer, and centrifuge the solution at 12,000xg for 15 min at 4C. The starch particles will be held in the pellet, while the nuclei and cell wall debris should float on top of the Percoll solution. This layer can be removed with a cut-off pipette and transferred to an appropriate tube (either 0.2ml or larger if needed at this time).
- Return to the main protocol at **step 11**.

**NOTE 6:** For the inexperienced researcher it is recommended to estimate the concentration of nuclei at this stage. At least  $10^6$  intact nuclei are needed for downstream processing. Take 1µl of resuspended nuclei, mix it with DAPI solution, and examine with a haemocytometer. This can be done typically within 10–15 min, during which time the remaining nuclear resuspension is left on ice.

**NOTE 7:** Dilute from supplied 10xNEB buffer. If another enzyme is used for digestion then use appropriate enzyme buffer.

**NOTE 8:** Consider the need to process appropriate controls. In this experiment the aliquot is split into 3 samples to address these controls:

1. An undigested control. The sample is not digested by DpnII as occurs in **Step 17**.
2. A control for the 'filling-in' reaction that takes place in **Step 19**. Samples in this control should preferentially re-ligate the DNA digested by DpnII in **Step 17** and will not ligate adjacent samples as in the regular protocol.

**NOTE 9:** Prepare a mastermix without Klenow fragment and then add enzyme individually to each tube. This is a crucial portion of the protocol that fills-in digested fragments, allowing for the blunt end ligation that occurs in **Step 20**.

**NOTE 10:** Described is the reaction for a blunt-end ligation. For a blunt-end ligation includes a concentration of ATP that is 10% of the value for sticky-end ligation.

**NOTE 11:** If a 6-cutter enzyme is used for chromatin digestion (*not DpnII*), the input DNA amount can be increased to 10 µg to account for less available ligation junction than are present in chromatin digested by a 4-cutter such as DpnII.

**NOTE 12:** Sonication parameters can be adjusted according to the DNA size of input sample. Parameters for CovarisS220 sonicator: Duty cycle 10, Intensity 5, Cycles per burst 200, Time 50s. <https://www.covaris.com/s220-focused-ultrasonicator-500217>

**NOTE 13:** It is recommended to use column purification as this will remove any additional excess polysaccharide in the sample.

**NOTE 14:** The concentration of AMPure XP beads that is added to a reaction controls the size of the DNA molecules that are removed. It is very important that beads are calibrated prior to use so that the correct amount is added and therefore the correct size of DNA molecules are retained or removed. <https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/pcr>

**NOTE 15:** AMPure XP beads here bind to DNA fragments >500bp. Note that at the end of this incubation step, it is the supernatant, which contains DNA fragments < 500bp, that is used for downstream library prep.

**NOTE 16:** AMPure XP beads here bind to DNA fragments >300bp.

**NOTE 17:** Treating with T4 polymerase will remove biotin-labeled cytosine residues from unligated DNA, which are present at the end of these DNA molecules. By supplying dTTP and dATP, the 3' to-5' exonuclease activity of T4 DNA polymerases is inhibited selectively so that bases 5' upstream of the terminal biotin-labeled cytosine are protected from removal. The choice of deoxynucleotide triphosphates used is dependent on the restriction enzyme that was used to digest the genomic DNA. For example, if the restriction enzyme is HindIII, dGTP and/or dATP but not dTTP should be supplied.

**NOTE 18:** Information about this Library Prep Kit for Illumina sequencing can be found here: <https://international.neb.com/products/e7805-nebnext-ultra-ii-fs-dna-library-prep-kit-for-illumina#Product%20Information>

If a different method of sample preparation is used either for Illumina or other next generation sequencing platform then the researcher will need to prepare their samples according to the specifics of that platform.

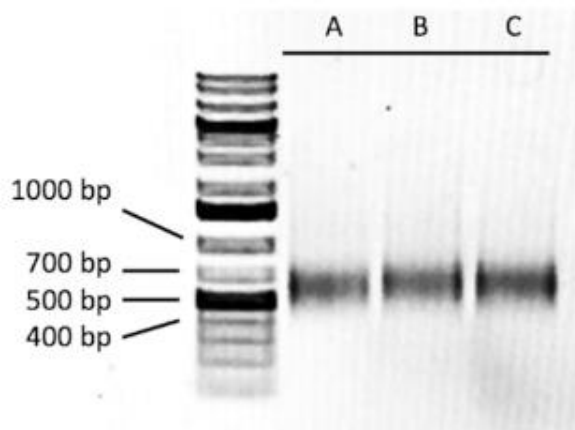
**Note 19:** In **Step 43**, the AMPure beads are mixed with DNA with a 0.8:1 ratio. This is not for a size selection purpose as due to a considerable amount of glycerol introduced during the adaptor ligation step, here nearly all DNA will be precipitated and recovered.

**NOTE 20:** This protocol uses the NEBNext<sup>®</sup> Ultra<sup>™</sup> II Library Prep system but other PCR reagents can be used in which case the specifics of those protocols should be followed. <https://international.neb.com/applications/ngs-sample-prep-and-target-enrichment/illumina-library-preparation/nebnext-ultra-ii-dna-library-prep>

**NOTE 21:** Suggested PCR cycles for analysis of Hi-C library (reproduced with permission from Chang Liu).

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	3–15*
Annealing/Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

**NOTE 22:** After analysis on an agarose gel a successful amplified library appears as a homogeneous smear between 400 and 700 bp. Gel example taken from Wang and Liu (2020).



**NOTE 23:** Biotin beads won't interfere the AMPure XP beads, you can directly do size selection

## 5. References

Ariel F, Lucero L, Christ A *et al* (2020) **R-Loop Mediated transaction of the APOLO Long Noncoding RNA**. *Mol Cell*. doi: 10.1016/j.molcel.2019.12.015.

Concia L, Veluchamy A, Ramirez-Prado JS *et al* (2020) **Wheat chromatin architecture is organized in genome territories and transcription factories**. *Genome Biol*. doi: 10.1186/s13059-020-01998-1

Dekker J, Rippe K, Dekker M, Kleckner N (2020) **Capturing chromosome conformation**. *Science*. doi: 10.1126/science.1067799

Dong P, Zhong S (2020) **Characterization of Plant 3D Chromatin Architecture, In Situ Hi-C Library Preparation, and Data Analysis**. *Methods Mol Biol*. 2020;2093:147-157. doi: 10.1007/978-1-0716-0179-2\_11

Dong P, Tu X, Li H *et al* (2020) **Tissue-specific Hi-C analyses of rice, foxtail millet and maize suggest non-canonical function of plant chromatin domains**. *J Integr Plant Biol*. doi: 10.1111/jipb.12809

Dong P, Tu X, Chu PY *et al* (2020a) **3D Chromatin Architecture of Large Plant Genomes Determined by Local A/B Compartments**. *Mol Plant*. doi: 10.1016/j.molp.2017.11.005

Grob S, Schmid MW, Luedtke NW, Wicker T, Grossniklaus U (2020) **Characterization of chromosomal architecture in Arabidopsis by chromosome conformation capture**. *Genome Biol*. doi: 10.1186/gb-2013-14-11-r129

Karaaslan ES, Wang N, Faiß N *et al* (2020) **Marchantia TCP transcription factor activity correlates with three-dimensional chromatin structure**. *Nat Plants*. doi: 10.1038/s41477-020-00766-0

Liu C (2017) **In Situ Hi-C Library Preparation for Plants to Study Their Three-Dimensional Chromatin Interactions on a Genome-Wide Scale**. *Methods Mol Biol*. doi: 10.1007/978-1-4939-7125-1\_11

Montgomery SA, Tanizawa Y, Galik B *et al* (2020) **Chromatin Organization in Early Land Plants Reveals an Ancestral Association between H3K27me3, Transposons, and Constitutive Heterochromatin**. *Curr Biol*. doi: 10.1016/j.cub.2019.12.015

Wang N, Liu C (2020) **Study of Cell-Type-Specific Chromatin Organization: In Situ Hi-C Library Preparation for Low-Input Plant Materials**. *Methods Mol Biol*. doi: 10.1007/978-1-0716-0179-2\_9

Wei Q, Wang J, Wang W *et al* (2020) **A high-quality chromosome-level genome assembly reveals genetics for important traits in eggplant**. *Hortic Res*. doi: 10.1038/s41438-020-00391-0

Wu H, Zhao G, Gong H *et al* (2020) **A high-quality sponge gourd (*Luffa cylindrica*) genome**. *Hortic Res*. doi: 10.1038/s41438-020-00350-9



<https://www.brookes.ac.uk/indepth/indepth-academy/>

Yang J, Deng G, Lian J *et al* (2020) **The Chromosome-Scale Genome of Melon Dissects Genetic Architecture of Important Agronomic Traits.** *iScience*. doi: 10.1016/j.isci.2020.101422