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Hi-C library construction from young Maize leaves

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In a recent study we constructed a high-quality chromosome-level reference genome for the maize cultivar Dan340 by combining PacBio long HiFi sequencing reads, Illumina short reads and chromosomal conformational capture (Hi-C) sequencing reads.

A Hi-C library was constructed using young leaves following previously published procedures with slight modifications. See the source paper and the following protocol.

Belton JM, McCord RP, Gibcus JH, Naumova N, Zhan Y, Dekker J (2012). Hi-C: a comprehensive technique to capture the conformation of genomes.. Methods (San Diego, Calif.). https://doi.org/10.1016/j.ymeth.2012.05.001

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protocol ,

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1 Approximately **5 g** leaf samples from seedling were cut into minute pieces and cross-linked by 4% formaldehyde solution at room temperature in a vacuum for **00:30:00** . 30m

2 Each sample was mixed with an excess of **2.5 Molarity (M)** glycine to quench the crosslinking reaction for **00:05:00** and then placed on ice for **00:15:00** . 20m

3 The cross-linked DNA was extracted and then digested for **12:00:00** with 20 units of DpnII restriction enzyme (NEB) at **37 °C** , and the resuspended mixture was incubated at **65 °C** for **00:20:00** to inactivate the restriction enzyme. 12h 20m

4 The sticky ends of the digested fragments were biotinylated and proximity ligated to form enriched ligation junctions and then ultrasonically sheared to a size of 300 - 600 bp.

5 The biotin-labelled DNA fragments were pulled down and ligated with Illumina paired-end adapters, and then amplified by PCR to produce the Hi-C sequencing library.

6 The library was sequenced using an Illumina HiSeq X Ten platform with 2 × 150 bp paired-end reads (Illumina, San Diego, CA, USA).

7 After removing low-quality sequences and trimming adapter sequences, we had 304.37 Gb (approximately 130×) of clean data generated. This is then used for genome assembly.

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