

Improving plant genome assemblies with Pore-C

High-quality plant genome sequences are critical for research into plant evolution, breeding, and conservation. However, plant genomes can be large and highly repetitive, making the process of genome assembly difficult using traditional technologies.

Pore-C is an end-to-end workflow combining chromatin conformation capture (3C) with long nanopore sequencing reads, providing contact information that allows existing assemblies to be scaffolded to high contiguity. Whilst traditional 3C methods typically only detect pairwise interactions between two genomic loci, Pore-C generates higher-order contact information from multiple genomic loci. The method is amplification-free, enabling access to GC-rich and repetitive genomic regions. Epigenetic modifications are also preserved in the long nanopore reads and can be characterised alongside nucleotide sequence. Together with high-output PromethION™ Flow Cells, highly contiguous, chromosome-scale plant genome assemblies are now possible.

Here we present a complete workflow for the scaffolding of a plant genome assembly to the chromosome scale.

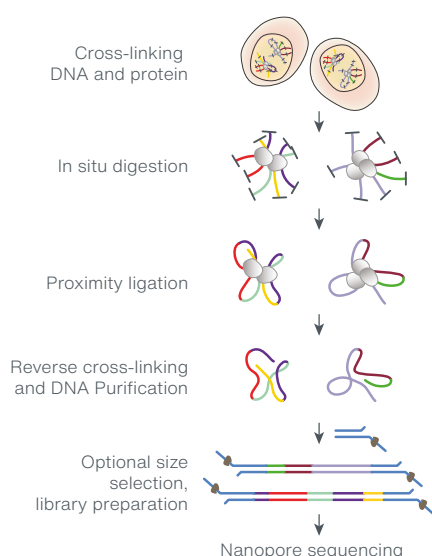


THE PORE-C WORKFLOW: cross-linking, DNA extraction, and library preparation

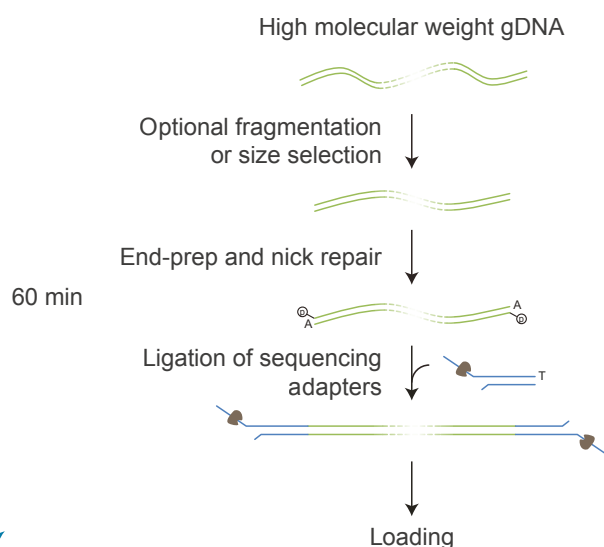
Find out more about Oxford Nanopore sequencing kits:
store.nanoporetech.com/sample-prep.html

Starting from a plant leaf sample, the plant Pore-C workflow begins with cross-linking, digestion, and proximity ligation: a process that fixes loci that are close together in 3D space, but not necessarily in the linear genomic sequence. After reverse cross-linking, the sample is then purified. To maximise the number of sequencing reads containing contacts, we recommend using SPRI beads to size select for ligated fragments above ~1.5–2 kb prior to library preparation. The Pore-C library is then prepared using the **Ligation Sequencing Kit**. However, if the yield of extracted DNA is <1 µg, we recommend using the **Rapid PCR Barcoding Kit** to maximise throughput in sequencing.

Pore-C workflow



Ligation Sequencing Kit workflow



For detailed guidance through the Pore-C workflow, including cross-linking, DNA extraction, and library preparation, visit: community.nanoporetech.com/info_sheets/restriction-enzyme-pore-c

SEQUENCING: generating high yields of long reads using PromethION

To scaffold an existing plant genome assembly to the chromosome level, we recommend sequencing Pore-C libraries on PromethION Flow Cells, to a minimum depth of 20x; for many plant genomes, this can be achieved by sequencing a Pore-C library on one PromethION Flow Cell for 72 hours.

Throughput can be maximised by performing a flow cell wash and loading fresh library approximately every 18 hours using the **Flow Cell Wash Kit**.



Find out more about PromethION sequencing devices:
nanoporetech.com/products/promethion

ANALYSIS: selecting assembly tools

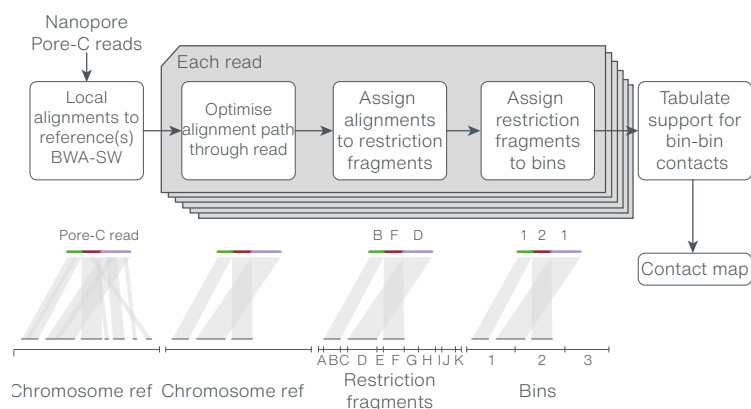
We recommend our comprehensive Pore-C Snakemake pipeline, which is available on GitHub, for analysis.

This workflow, which requires familiarity with the command line, creates a contact map using the Pore-C reads and aligns reads against a reference genome.

We then recommend the tool Salsa2¹ to utilise this information to orient contigs, allowing for assembly scaffolding and correcting previous misassemblies. Using this method, a highly contiguous, chromosome-scale plant genome assembly can be produced.

View the Pore-C analysis pipeline on GitHub:
github.com/nanoporetech/pore-c

Pore-C Snakemake pipeline



Find out more at: nanoporetech.com/applications/investigation/chromatin-conformation

References:

1. Ghurye, J. et al. PLoS Comput Biol. 15(8):e1007273 (2019).

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