Agilent Case Study: Fragment Analyzer system

New DNA Extraction Method for Long-Read Sequencing in Plant Tissues at the University of Illinois

Novel Genome Assemblies for Plants

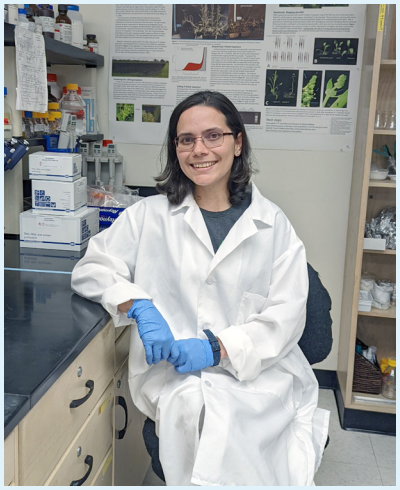
The University of Illinois Urbana-Champaign researchers Dr. Dessireé Zerpa-Catanho, Dr. Ray Ming, and Dr. Alvaro Hernandez, along with members of their research teams, recently published a unique DNA extraction method. It was designed for the preparation of high-purity, high-molecular-weight (HMW) plant DNA suitable for long-read Oxford-Nanopore next-generation sequencing (NGS).[1]

The method will be used to generate long- and ultralong-reads which will then be used for the novel genome assembly of species in the Caricaceae, a family of tropical flowering plants including papaya and other edible fruits. The information provided by the genome assembly will further the study of this economically important plant family's sex determination pathways and its evolutionary history.

Long DNA fragments from plant tissues are important for producing novel genome assemblies. "The problem with de novo genome assemblies for plants is that they contain a lot of repetitive DNA elements and longer fragments are able to resolve these repeats. But shorter fragments make it basically impossible to obtain a good assembly," asserted Dr. Zerpa-Catanho.

High-Molecular-Weight DNA from Plant Tissues

Because it was necessary to generate long DNA fragments, the research team knew they needed to develop a procedure specific for plant tissues. The team found it challenging to extract DNA of the right quality and quantity that was adequate for long-read sequencing. One important consideration that helped was the use of fresh plant leaves. By using young plant tissues, the research team noticed an improvement in the purity of the HMW DNA. "We prefer to use fresh, young leaves, without any signal of disease because adult leaves can contain a higher content of contaminants like polysaccharides, polyphenols and others," said Dr. Zerpa-Catanho. In addition to using fresh leaves for sampling, the research team implemented a purification step prior to sequencing to address any residual impurities such as proteins, phenols, or other organic compounds



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DNA quantity and quality are other important factors for long-read NGS success. "If you don't get enough nuclei from the first extraction, you will probably not get enough DNA," Dr. Zerpa-Catanho said. To help produce a large quantity of high-quality fragments, it is critical to be careful with the sample and use specialized tools such as wide-bore pipette tips throughout the workflow. She conveyed that it is key to, "handle the sample gently during the extraction because you won't want to have all of your DNA fragmented if you are doing long-read or ultralong-read DNA sequencing." The quality and quantity of the extracted DNA were then tested using various quality assessments.

Multiple Quality Tests with Different Goals

By selecting among different sample quality control procedures, the team was able to ensure that their DNA extractions contained fragments of DNA that were highly pure and of sufficient length for long- and ultralong-read sequencing. The team used UV-Vis spectrophotometry and fluorometry to measure DNA quantity and identify contaminants. Dr. Zerpa-Catanho verified the presence of long fragments in the samples by running the DNA on an agarose gel. Although it was possible to detect long DNA fragments, ultralong DNA fragments, and large quantities of small DNA fragments on the agarose gel, it was difficult to see small quantities of shorter DNA fragments (<50 kb).

To better discern whether the DNA samples contained smaller fragments, she collaborated with Dr. Alvaro Hernandez, Director of the University of Illinois Urbana-Champaign DNA Services Facility. Dr. Hernandez recommended using the Agilent Fragment Analyzer system. Dr. Zerpa-Catanho noted, "Dr. Hernandez suggested using the Fragment Analyzer just to make sure that our DNA was not fragmented or degraded; we could see smaller fragments that way." Because the data generated by the Fragment Analyzer system is presented in an electropherogram, small quantities of shorter fragments are easy to see, even if those short fragments are spread out. The objective data from the Fragment Analyzer system were displayed in a report, which enabled the team to ascertain whether the sample was of acceptable quality and length for long-read sequencing. The results confirmed that their DNA extraction method was performing as intended.

Learn more about the Agilent Fragment Analyzer system at: **www.agilent.com/genomics/fragment-analyzer**

Just the Beginning for Plant Genome Assembly

The extraction method developed by these researchers can produce high-purity samples which yield an average read distribution of 25 kilobases. These reads are then used for novel genome assembly of different species in the Caricaceae family. In addition, they have been able to save resources while producing HMW DNA. Dr. Zerpa-Catanho reported, "The lab was able to extract high-molecular-weight DNA in a cheaper way. When you send your samples to service labs you end up paying a lot for extractions."

Although Dr. Zerpa-Catanho and the team originally designed this extraction method to obtain suitable DNA from plant tissues for Oxford-Nanopore long-read sequencing, they envision that the procedure will be used in future applications such as PacBio or Illumina sequencing technologies. The team also anticipates that the method will work well for the analysis of tissue from other plant species in addition to Caricaceae. There are many species that still have not had their genomes assembled, but Dr. Zerpa-Catanho affirmed, "every day we have more and more genome assemblies coming out for plants."

Conclusion

The research team overcame several technical challenges and successfully developed a robust and cost-effective DNA extraction method for plant tissues. This method delivers high-purity HMW DNA intended for Oxford-Nanopore long-read and ultralong-read sequencing. The quality and quantity of the DNA is assessed prior to sequencing using distinct quality control techniques, including assessing the overall size of HMW DNA and the presence of small DNA fragments using the Agilent Fragment Analyzer system. The data generated through nanopore sequencing can be used for the novel genome assembly of plant species in the Caricaceae family.

Reference

1. Zerpa-Catanho, D; Zhang, X; Song, J; Hernandez, AG; Ming, R. Ultra-Long DNA Molecule Isolation from Plant Nuclei for Ultra-Long Read Genome Sequencing. *Cell* **2021**, *183*, 875-889.e117. https://doi.org/10.1016/j.xpro.2021.100343

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