

Digging deeper into DNA: An efficient method to sequence chloroplast genomes

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To fully understand a plant's nuclear genome, scientists must also study two other genomes found within plant cells—in the "powerhouse" mitochondria and in the photosynthesizing chloroplast organelles.

Researchers from the Instituto Nacional de Investigación Agropecuaria (National Institute of Agricultural Research) in Uruguay have developed a chloroplast genome-sequencing strategy to facilitate this research. The new method could unlock a wealth of untapped chloroplast genome sequence data that can be applied to evolutionary studies.

"Because it is difficult to remove plastid DNA from nuclear DNA, many nuclear genomes available in public repositories contain enough plastid DNA to assemble their corresponding genomes," explains lead researcher Silvia Garaycochea.

According to the researchers, several fragments and, in some cases, nearly entire copies of the [chloroplast genome](#) may be found within the nuclear genomes of [plants](#). This is because genetic material from chloroplasts has been continuously transferring to the nucleus through years of evolution. Chloroplasts are understood to have originated from prokaryotes that were engulfed by eukaryotes millions of years ago.

The new method by Garaycochea and colleagues will enable researchers to strategically analyze this whole-genome sequence data and assemble the chloroplast genome for their plant of interest. It is less costly and less time consuming than other methods. Certain tedious lab procedures—such as prior plastid DNA isolation, plastid DNA

enrichment, and reliance on a reference genome—are not required.

"For chloroplast genome recovery from total DNA sequence data, the deliberate identification of reads that represent chloroplast DNA inserts into the [nuclear genome](#) allowed us to attain a higher-quality chloroplast genome assembly in a time- and cost-effective way," Garaycochea explains.

Using the new method, Garaycochea et al. extracted whole-genome [sequence data](#) from red rice (*Oryza sativa* L.) and produced a complete chloroplast genome, which is now available on GenBank. The full study is available in a recent issue of *Applications in Plant Sciences*.

"We used weedy rice as a model plant. This choice allowed us both to obtain a chloroplast sequence of interest for research, and to take advantage of the wealth of available information to validate our results," says Garaycochea.

While analyzing the red rice genome, Garaycochea et al. found that plastid DNA transfer is more frequent than previously thought. These transfer events are highly valuable because they can reveal new information about the evolutionary relationships within and between species.

"Depending on the time of DNA transfer events, these sequences may retain different degrees of similarity to the original plastid genome," says Garaycochea. "These nuclear DNA segments of chloroplast origin can provide valuable evolutionary information."

Thousands of plant species have had their genomes sequenced, but without organelle genomes, nuclear genomes are only one piece of the DNA puzzle. In addition to new evolutionary discoveries, the chloroplast genome can offer a deep look at important plant processes that are

tightly linked to today's changing environment, such as the impacts of excessive heat and drought on photosynthetic productivity.

More information: Silvia Garaycochea et al. A Strategy to Recover a High-Quality, Complete Plastid Sequence from Low-Coverage Whole-Genome Sequencing, *Applications in Plant Sciences* (2015). [DOI: 10.3732/apps.1500022](https://doi.org/10.3732/apps.1500022)

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