Breaking down DNA by genome

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New DNA sequencing technologies have greatly advanced genomic and metagenomic studies in plant biology. Scientists can readily obtain extensive genetic information for any plant species of interest, at a relatively low cost, rapidly accelerating the pace of genome sequencing.

However, since plant tissues harbor three separate genomes (nuclear, chloroplast, and mitochondrial), it can often be challenging to isolate the particular genome of interest from extracted DNA samples. Sequencing DNA containing all three genomes therefore results in a considerable amount of wasted data, for example, if only the chloroplast genome is desired for the study.

Methods exist to isolate particular genomic regions, but each of these has drawbacks. For example, some protocols require extensive lab work, while others (e.g., long-range PCR and hybrid enrichment) require prior knowledge of the genomic regions of interest.

A new method by researchers from New England Biolabs and New Mexico State University provides plant biologists with a quick and simple approach for separating plant nuclear DNA from organellar DNA for genomic and metagenomic studies. The approach, published in the November issue of *Applications in Plant Sciences*, targets the methyl-CpG-binding domain, following a similar method applied for genomic studies of humans.

The method relies on differences in CpG methylation between nuclear and organellar (i.e., chloroplast and mitochondrial) genomes in plants.

Compared to the nuclear genome, the chloroplast and mitochondrial genomes essentially lack CpG methylation (i.e., the addition of methyl groups to sites in the genome where cytosine and guanine occur side by side).

Given these different methylation patterns, the researchers used specialized magnetic beads that hybridize with methyl-CpG-rich DNA regions in an attempt to separate nuclear DNA from organellar DNA in total genomic DNA samples. They then sequenced the methyl-enriched portion and the methyl-depleted portion separately. They found that the methyl-enriched sample contained a considerable increase in concentration of nuclear DNA, while the methyl-depleted sample contained an increased concentration of organellar DNA.

Dr. Donovan Bailey, senior author of the study and professor at New Mexico State University, said this approach has several advantages over previously established methods for enriching either nuclear or organellar DNA for genome sequencing.

"Our primary perceived benefit includes the development of a means of partitioning DNA by genomic origin when one has no prior knowledge of the genomes being studied, other than the domain of origin—nuclear, organellar, or prokaryote. Furthermore, not requiring extensive starting material and the speed are benefits relative to some methods."

According to Bailey, this approach can also be used to target genomes of endophytes (i.e., fungi that live in plants) and prokaryotic parasites in plant DNA samples. Endophyte genomes undergo CpG methylation, while prokaryotic genomes do not, making it easy to sequence either of these along with the particular plant genome(s) of interest. This will provide researchers with greater insight on the diversity of other eukaryotes and prokaryotes living inside plant tissues.

Although this study focused on flowering plants, Bailey said the approach will likely work well across other major plant groups (e.g., ferns, gymnosperms).

More information: Erbay Yigit, David I. Hernandez, Joshua T. Trujillo, Eileen Dimalanta, and C. Donovan Bailey. Genome and metagenome sequencing: Using the human methyl-binding domain to partition genomic DNA derived from plant tissues. Applications in Plant Sciences 2(11): 1400064. DOI: 10.3732/apps.1400064

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