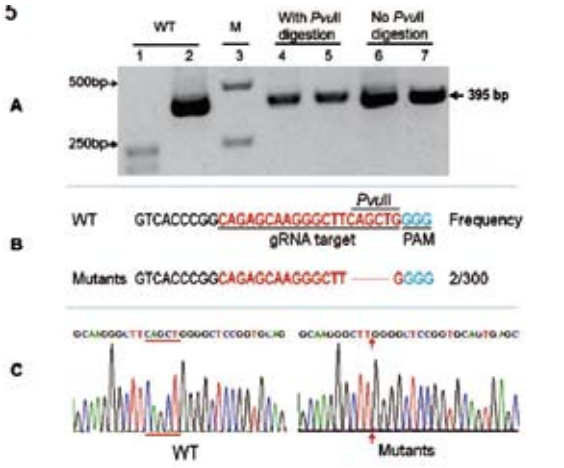
Novel Genome Editing Platform for Industrial Oleaginous Microalgae

Microalgae are organisms that can use sunlight to capture and assimilate atmospheric CO_2. They then store the solar energy and CO_2 in the form of energy-dense molecules such as triacylglycerol (TAG), which can be readily converted to oil. Therefore, the interest in microalgae as a scalable solution for clean fuel production and CO_2 sequestration has been growing.

However, the paucity of genetic and genome engineering tools has greatly hindered the mechanistic studies and molecular breeding of energy microalgae. For example, *Nannochloropsis* spp. is a group of industrial oleaginous microalgae that can be cultured in large scale with sea water. They are of industrial interest due to their ability to grow rapidly under a wide-range of scales, synthesize large amounts of TAGs and high-value polyunsaturated fatty acids (PUFAs) and tolerate broad environmental and culture conditions. But the lack of reverse genetic engineering tools has hindered efforts to genetically improve key traits such as the efficiency and capacity of carbon fixation and oil production.

In a new study published in the *Plant Journal*, WANG Qintao, LU Yandu and their coworkers from the Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences established a novel genome editing platform for the industrial oleaginous microalgae *Nannochloropsis oceanica*. The platform employs a well-known targeted gene knockout method based on the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9. The researchers codon-optimized the Cas9 protein and designed the guide RNA, then managed to coexpress the gRNA and Cas9 protein in *Nannochloropsis oceanica*. Next-generation sequencing revealed that the method successfully generated a mutation profile that was dominated by 5-base deletion situated precisely at the gRNA targeted site. Knockout of endogenous nitrate reductase gene via this approach led to growth failure under nitrate ($NaNO_3$), yet uncompromised growth under ammonium (NH_4Cl). This is one of the first demonstrations of genome editing in industrial oleaginous microalgae.



Genotypic validation of the nitrate reductase mutants generated by Cas9/gRNA-mediated genome-editing.

The CRISPR/Cas9-based genome-editing method has expanded the reverse genetics toolbox of industrial oleaginous microalgae, and introduced numerous possibilities in the systems and synthetic biology of microalgae-based carbon dioxide capture and conversion. For example, functional probing and validation of every coding- and noncoding-element on the *Nannochloropsis* genome now become possible. Combined with other platforms at the institute such as single-cell Raman imaging, Raman screening and sequencing, this genome editing platform will enable the establishment of the genotype and phenotype links with a new level of precision, breadth and throughput. This will lay the foundation for the design and construction of microalgal traits via precision surgery of the genome.

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