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CRISPR-Cas ribonucleoprotein mediated homology directed repair for efficient targeted genome editing in microalgae *Nannochloropsis oceanica* IMET1

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Abstract

Microalgae *Nannochloropsis spp* gained scientific attention due to their increased capabilities for producing Polyunsaturated fatty acids (PUFA) and Triacylglycerol (TAG). The high production cost of these microalgae is a major bottleneck in commercializing them. However, this could be solved through metabolic engineering which requires reliable genome-editing techniques. CRISPR-Cas system has been implemented for successful genome editing in *Nannochloropsis spp*. Alternative to the previous reports, we use Cas9/Cas12a ribonucleoprotein (RNP) for Homology Directed Repair (HDR) based genome editing in *N. oceanica*.

Initially, we attempted to obtain Nitrate reductase (NR) mutants by homologous recombination (HR) and antibiotic selection in *N. oceanica* IMET1. As DSB induction was reported to enhance HR by HDR, a Cas9 RNP targeting NR gene was co-transformed with the same editing template to enhance the frequency of mutants among the transformants. Subsequently, the potential of other Cas12a RNPs was tested to find the best Cas protein to be applied in *N. oceanica*.

The approach based on HR and antibiotic selection did not yield any NR mutants. DSB induction by Cas9 RNP in combination with HDR resulted in approximately 70% mutants among the transformants. In a similar approach with Cas12a variants, FnCas12a produced up to 92% mutants. LbCas12a showed efficiencies similar to Cas9 while AsCas12a yielded the least number of mutants.

We report for the first time in *Nannochloropsis spp*, a Cas ribonucleoproteins (RNP) based HDR as a genome editing strategy in *N. oceanica* to successfully knockout the NR gene. Upon comparing multiple Cas12a variants, we found that Cas12a from *Francisella novicida* (FnCas12a) is the best Cas variant for HDR based gene editing in *N. oceanica* IMET1 and AsCas12a is the least effective. Attempts to obtain multiplexed DNA free markerless gene editing in *N. oceanica* are ongoing.

Biography:

After completing his bachelor degree in Biotechnology and Biochemical Engineering from Sree Chitra Thirunal College of Engineering, Kerala University, India, Mihris moved to Wageningen for pursuing his MSc degree in Cellular and Molecular Biotechnology. During the course of his MSc degree he developed a fascination on potential of genetically modified microbes and the techniques used for developing mutants. This led to his MSc thesis in the Bacterial genetics lab in Microbiology department where he worked on Metabolic engineering of thermophilic bacteria Bacillus smithii and his work was awarded the UFW-KLV thesis award from the Wageningen University. Later, on completion of his masters, he works as a PhD candidate in the same department under the supervision of Prof. John van der Oost and Dr. Maria Barbosa where his research focus on developing CRISPR-Cas based genome editing tools for microalgae.