https://doi.org/10.17816/ecogen568609

CRISPR/Cas based genome editing in microalgae

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CRISPR/Cas systems are presently the most attractive genome editing technology, that is widely used for genetic engineering of various crops and industrial microorganisms. Currently, application of the CRISPR/Cas based genome editing promises advances in microalgae biotechnology aimed at boosting the output of biofuels and valuable bioactive compounds. However, algae remain relatively complex objects for genetic manipulation [1]. The main problems are associated with the need of a species-oriented approach when creating a transformation toolbox due to the peculiarities in the structure of membranes and the cell wall of a particular taxon. The proper selection and design of a CRISPR construct is also required due to the possible presence of a powerful silencing system against introduced genetic constructs in the cell. These difficulties explain the low efficiency of microalgae transformation and the meager list of successfully edited species [1, 2].

The first instance of genome editing in microalgae using CRISPR/Cas was reported in *Chlamydomonas reinhardtii* P.A. Dang [3]. To date, four transformation methods (*Agrobacterium*-mediated, particle bombardment, glass beads agitation, electroporation) have been successfully used for editing (knock-in and knock-out) the *C. reinhardtii* genome with two types of CRISPR constructs (plasmid and ribonucleoprotein). The developed protocols make it possible to achieve high efficiency of genomic editing — for example, in our study it varied from 10.6% to 68.8% [4]. These benefits along with completely sequenced genome, well-studied genetics, accessibility and haplontic life cycle makes *C. reinhardtii* an outstanding model organism for CRISPR/Cas application in microalgae research [5].

Keywords: CRISPR/Cas; genome editing; transformation toolbox; CRISPR construct delivery; microalgae; GMOs.

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