

Assessment of genomic changes in a CRISPR/Cas9 *Phaeodactylum tricornutum* mutant through whole genome resequencing

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ABSTRACT

The clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 system, co-opted from a bacterial defense natural mechanism, is the cutting edge technology to carry out genome editing in a revolutionary fashion. It has been shown to work in many different model organisms, from human to microbes, including two diatom species, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. Transforming *P. tricornutum* by bacterial conjugation, we have performed CRISPR/Cas9-based mutagenesis delivering the nuclease as an episome; this allowed for avoiding unwanted perturbations due to random integration in the genome and for excluding the Cas9 activity when it was no longer required, reducing the probability of obtaining off-target mutations, a major drawback of the technology. Since there are no reports on off-target occurrence at the genome level in microalgae, we performed whole-genome Illumina sequencing and found a number of different unspecific changes in both the wild type and mutant strains, while we did not observe any preferential mutation in the genomic regions in which off-targets were predicted. Our results confirm that the CRISPR/Cas9 technology can be efficiently applied to diatoms, showing that the choice of the conjugation method is advantageous for minimizing unwanted changes in the genome of *P. tricornutum*.

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INTRODUCTION

Diatoms are photosynthetic unicellular eukaryotes responsible for 20% of the global carbon fixation and present in marine and freshwater habitats with roughly 100,000 different species, all characterized by the presence of a silica shell and extraordinary richness of different cell shapes (*Falkowski, Barber & Smetacek, 1998; Armbrust, 2009; Mann & Vanormelingen, 2013*). These organisms have been attracting scientific interest for a long time for their ecological role and extraordinary biodiversity, for their complex evolutionary history and physiological properties, for the capability of adaptation to considerably diverse environments thanks to unexpected metabolic abilities, and, more