DOI: https://doi.org/10.17816/ecogen112332

Optimization of CRISPR/Cas9 method for transgenesis of model microalgae *Chlamydomonas reinhardtii*

Pavel A. Virolainen [1], Elena M. Chekunova [2]

[1] N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Saint Petersburg, Russia;
[2] Saint Petersburg State University, Saint Petersburg, Russia

In this work we knocked out the *LTS3* gene of the microalgae *Chlamydomonas reinhardtii* using the TIM technique optimized for the available equipment. We achieved transformation efficiency of 68.8%, knockout of this gene lead to the death of *C. reinhardtii* cells after several division cycles.

The creation and study of genetically modified organisms in fundamental research allows a deeper understanding of the basic processes in the cells with the prospect of further applying this knowledge in practice. Microalgae are an interesting object for genetic engineering because of the great prospects for their application in biotechnology, but in almost every case it is necessary to develop new strategies and transformation methods for the introduction of genetic constructs into the cell. CRISPR/Cas revolutionized the field of genome editing due to its simplicity, efficiency and accuracy compared to previously used methods, which over time simplified the development of protocols [1]. Currently, the most effective method of transformation is TIM (Targeted Insertional Mutagenesis) [2], developed for the microalgae *Chlamydomonas reinhardtii* P.A. Dang. – model object of photosynthesis genetics.

To test and optimize the TIM technique [2] in our lab, we carried out a knockout of the *LTS3* gene, a transcriptional activator of chlorophyll biosynthesis genes in heterotrophic conditions [3].

We used glass beads agitation and electroporation ("Gene Pulser Xcell", Bio-Rad, USA) methods in order to introduce into *C. reinhardtii* cells of the CC-125 (*wt*, *mt+*) strain the ribonucleoprotein complex SpCas9/sgRNA and double-stranded donor DNA with paromomycin resistance gene.

The effectiveness of transformation varied from 10.6% to 68.8%. Probably, the *LTS3* gene product plays a key role in the pathway of chlorophyll biosynthesis, since its knockout led to the death of *C. reinhardtii* cells after several division cycles.

The transformation protocol optimized for the equipment available in our lab can be further refined and used to study the functions of other *C. reinhardtii* genes.

Keywords: genetic engineering; genome editing; microalgae; *Chlamydomonas reinhardtii*; CRISPR/Cas; GATA transcription factors.

REFERENCES

1. Jeon S, Lim J-M, Lee H-G, et al. Current status and perspectives of genome editing technology for microalgae. *Biotechnology for Biofuels*. 2017;10:267. DOI: 10.1186/s13068-017-0957-z
2. Picariello T, Hou Y, Kubo T, et al. TIM, a targeted insertional mutagenesis method utilizing CRISPR/Cas9 in *Chlamydomonas reinhardtii*. *PLoS ONE*. 2020;15:5. DOI: 10.1371/journal.pone.0232594
3. Chekunova EM, Savelieva NV. LTS3 gene controls light-independent chlorophyll biosynthesis in green algae *Chlamydomonas reinhardtii*. *Ecological genetics*. 2010;8(2):35–44. (In Russ.) DOI: 10.17816/ecogen8235-44

AUTHORS' INFO

Pavel A. Virolainen, PhD Student. N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Saint Petersburg, Russia. SPIN: 6564-9350; e-mail: s.pasha98@yandex.ru

Elena M. Chekunova, Doctor of Science, Senior Teacher. Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 2788-6386; e-mail: elena_chekunova@mail.ru