Efficient transgene-free targeted DNA editing and replacement in green algae

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Genome editing nucleases are currently revolutionizing both molecular biology and industrial biotechnology. One of the latest additions to these nucleases is the RNA-programmable CRISPR/Cpf1 (Cas12a). CRISPR endonucleases induce targeted double-stranded DNA breaks, triggering cellular DNA repair pathways. Of these pathways, non-homologous end-joining (NHEJ) results in random insertions and deletions at the target site, whereas HR allows homology-directed, precise editing by using DNA repair templates. We recently demonstrated that single-step codelivery of CRISPR/Cpf1 ribonucleoproteins with single-stranded DNA repair templates (ssODN) results in precise and targeted DNA replacement with as much as ~10% efficiency in the green alga *Chlamydomonas reinhardtii* (Ferenczi *et al*., 2017). We generated sequence-specific mutations and an in frame gene tag without selection and transgenes. We will discuss the latest developments of this technology.

Ferenczi, A., Pyott, D. E., Xipnitou, A. and Molnar, A. Efficient targeted DNA replacement in *Chlamydomonas reinhardtii* using single-stranded oligodeoxynucleotides and Cpf1 ribonucleoproteins. *PNAS*, DOI: 10.1073/pnas.1710597114 (2017).