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Highly efficient genome editing and DNA replacement in green algae using programmable Cpf1 ribonucleoproteins and single-stranded DNA

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The biotechnological exploitation of algae is constrained by the limited number of genetic tools that are currently available for modulating nuclear gene expression. RNA-programmable CRISPR endonucleases induce targeted, double-stranded DNA breaks (DSB), and consequently trigger the cellular DNA repair mechanisms. Recent application of the genome editing CRISPR/Cas9 system to microalgae has yielded only low frequency random mutations at the target site due to the predominant activity of the non-homologous end-joining (NHEJ) DNA repair pathway. We found that single-step co-delivery of pre-assembled CRISPR/Cpf1 ribonucleoproteins with single-stranded oligodeoxynucleotide (ssODN) repair templates results in targeted DNA replacement with high efficacy in the green model algae *Chlamydomonas reinhardtii*. We generated sequence-specific mutations, nucleotide replacements and an in frame gene tag without selection and transgenes. We believe that harnessing the CRISPR/Cpf1-ssODN technology can pave the way to create the next generation of designer algae for basic and applied research as well as for the industry.

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