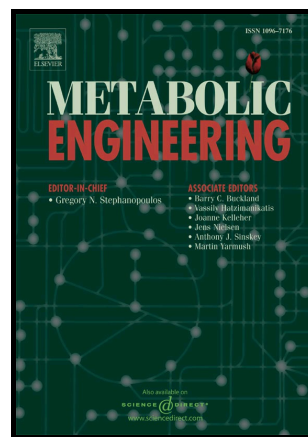


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Exploiting endogenous CRISPR-Cas system for multiplex genome editing in *Clostridium tyrobutyricum* and engineer the strain for high-level butanol production

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Abstract

Although CRISPR-Cas9/Cpf1 have been employed as powerful genome engineering tools, heterologous CRISPR-Cas9/Cpf1 are often difficult to introduce into bacteria and archaea due to their severe toxicity. Since most prokaryotes harbor native CRISPR-Cas systems, genome engineering can be achieved by harnessing these endogenous immune systems. Here, we report the exploitation of Type I-B CRISPR-Cas of *Clostridium tyrobutyricum* for genome engineering. *In silico* CRISPR array analysis and plasmid interference assay revealed that TCA or TCG at the 5'-end of the protospacer was the functional protospacer adjacent motif (PAM) for CRISPR targeting. With a lactose inducible promoter for CRISPR array expression, we significantly decreased the toxicity of CRISPR-Cas and enhanced the transformation efficiency, and successfully deleted *spo0A* with an editing efficiency of 100%. We further evaluated effects of

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