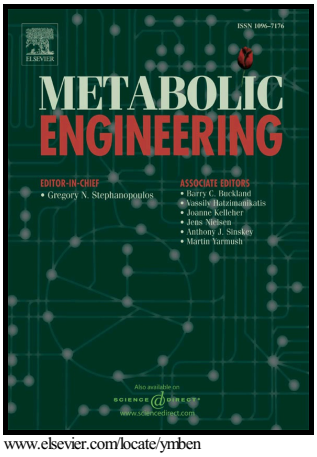

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PII: S1096-7176(18)30059-4
DOI: https://doi.org/10.1016/j.ymben.2018.03.007
Reference: YMBEN1361

To appear in: *Metabolic Engineering*

Received date: 7 February 2018
Revised date: 4 March 2018
Accepted date: 6 March 2018

Cite this article as: Jie Zhang, Wenming Zong, Wei Hong, Zhong-Tian Zhang and Yi Wang, Exploiting endogenous CRISPR-Cas system for multiplex genome editing in *Clostridium tyrobutyricum* and engineer the strain for high-level butanol production, *Metabolic Engineering*, https://doi.org/10.1016/j.ymben.2018.03.007

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

Jie Zhang[a], Wenming Zong[a,b], Wei Hong[a,c], Zhong-Tian Zhang[a], Yi Wang[a,d,*]

[a] Department of Biosystems Engineering, Auburn University, Auburn, AL 36849, USA

[b] School of Engineering, Anhui Agricultural University, Hefei 230036, China

[c] Key Laboratory of Endemic and Ethnic Diseases (Guizhou Medical University), Ministry of Education, Guiyang 550000, China

[d] Center for Bioenergy and Bioproducts, Auburn University, Auburn, AL 36849, USA

* Corresponding author: Department of Biosystems Engineering, Auburn University, 350 Mell Street, Auburn, AL, 36849 USA. Tel: 1-334-844-3503; Fax: 1-334-844-3530. yiwang3@auburn.edu

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