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Optimal CRISPR-cas9 system design for microbial cell factories

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Both bacteria and yeast have been used for different chemicals production and/or waste removal. To be used efficiently as microbial cell factories; metabolic engineering research is the winning card to improve them via genome editing tools. Many tools were emerged like zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), but the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system is leading the way now for this purpose. Despite of its successful and growing application in mammalian cells, it still encounters many problems that limit its application in microbial cell factories such as cas9 protein toxicity, absence of clear single guided-RNA (sgRNA) efficiency parameters and difficult vectors engineering, especially in new discovered microbial species. Here, we will focus in the basics for optimal design and construction of plasmids carrying cas9 gene and sgRNA, the solutions for cas9 toxicity and the choice of effective sgRNA. We will also show our results from its applications for production of succinic acid using *Escherichia coli*, lactic acid using *Saccharomyces cerevisiae* and desulfurization of natural gas and biogas using *Thioalkalivibrio versutus* via metabolic engineering of glycolysis and sulfur pathways.

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