CRISPR-based genome engineering of Corynebacterium glutamicum

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Corynebacterium glutamicum is an important industrial bacterial host for the production of amino acids. A new genome engineering tool is needed to accelerate the development of industrially capable strains. In this study, we reported a CRISPR/Cas9-coupled recombineering system as a strategy to knock out one or more genes in C. glutamicum. First, synthetic single stranded oligodeoxyribonucleotides targeting the desired genomic region are introduced by the recombinase (RecT). The CRISPR/Cas9 complex is then used to counter-select the negative mutants. The efficient curing system was designed to perform multiple gene engineering and to obtain plasmid-free strains. Using this system, metabolically engineered C. glutamicum strain was developed for γ-aminobutyric acid production. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea [NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557.