Metabolic engineering of *C. glutamicum* using CRISPR technology for the production of GABA

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Genome engineering of *Corynebacterium glutamicum*, an important industrial fermentative microorganism, relies on random mutagenesis and inefficient double crossover events. Here, we report a rapid genome engineering strategy to scarlessly knock out genes in *C. glutamicum* in sequential and iterative manner. Recombinase RecT is used to incorporate synthetic single-stranded DNA into the genome and CRISPR/Cas9 to counter-select negative mutants. The system was completed by CRISPR/Cas9 and RecT plasmids engineered for efficient curing such that multiple gene targets can be edited iteratively and final strains free of plasmids. Seven different mutants were constructed within two weeks, three of which demonstrated high level production of ɣ-aminobutyric acid, an industrially relevant chemical of much interest. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT through the National Research Foundation of Korea [NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557]. This work was further supported by Hanwha Chemical through KAIST-Hanwha Chemical Future Technology Institute.]