CRISPR/Cas9-based genome engineering of *Corynebacterium glutamicum* for high-level γ-aminobutyric acid production

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Genome editing in *Corynebacterium glutamicum*, an important industrial microorganism for amino acids production, have heavily been based on random mutagenesis and rather inefficient double crossover events. Here, we report a rapid genome engineering strategy to scarlessly knock out one or more genes in *C. glutamicum* in sequential and iterative manner. Recombinase RecT is used to incorporate synthetic single-stranded oligodeoxyribonucleotides into the genome and CRISPR/Cas9 to counter-select negative mutants. We completed the system by engineering the respective plasmids harboring CRISPR/Cas9 and RecT for efficient curing such that multiple gene targets can be done iteratively and final strains will be free of plasmids. To demonstrate the system, seven different mutants were constructed within two weeks to study the combinatorial deletion effects of three different genes on the production of γ-aminobutyric acid (GABA), an industrially relevant chemical of much interest as a precursor to polyamide-4. This genome engineering strategy will expedite metabolic engineering of *C. glutamicum*.
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