

[Back to article](#)**Table S1.** Primers to generate recombinant *Corynebacterium glutamicum* using CRISPR-cpf1 gene editing vector

pJY53Am_MCS	Fragment 1 (Cpf1 gene) Sense: 5' CTTTAGGTTCTCCCATGGTCATGACCTGAGCTGTTTACAATT 3' Anti-sense: 5' GGTTCTGTTCTCATGGCTCACGC 3' Fragment 2 (Rep101+ pBL1st) Sense: 5' CCTGCAGGCATGCAAGCTTAAGAG 3' Anti-sense: 5' GAAATGGTCTCCCATGGTGACCGCCTCGATGATCGCC 3' Fragment 3 (Amp gene) Sense: 5' CTTTAGGTTCTCCCATGGAAAGGGCCTCGTGATAC 3' Anti-sen: 5' CCAATCCACACATGGTACCACAGATGATTAATTGTAAACAGCTCAGGTCATGCTGA CAGTTA CCAATGCTTAATCAGTG 3'
Target DNA 1 set (crRNA + target DNA)	Sense: 5' CCTGCAGGCATGCAAGCTTAAGAG 3' Anti-sense: 5' GAAATGGTCTCGGATCCGAATTTCTACTGTTGTAGATGAGTTGCATACGCAT ACGCACTGCAATTTAAATAAAACGAAAGGCTCAGTCG 3'
Target DNA 2 set (crRNA + target DNA)	Sense: 5' GAAATGGTCTCACTAGTTTGACAGTAGCTCAGTCTAGGTATAATTCTAGAGAATTTCTACTGTTGTAGATGCGTCGATAATG 3' Anti-sense: 5' CTTTAGGTTCTCGGGCCATCGGCATTTTCTTTTTCGTTTCCCGGGCGCTGCCTATCA CATTATCGACGCATCTACAACAG 3'
	Experiment: After annealing two primers, the short DNA fragments were synthesized. The PCR products were digested with restriction enzymes and inserted into the pJY53Am_MCS vector.
pCold 1 vector	Construction of homologous arms and kanamycin selection marker gene.
LdhAp (Left arm)	Sense: 5' GAAATGGTCTCGGGCCGCTCCGCTCCACGGCTGCTC 3' Anti-sense: 5' GAAATGGTCTCGGTACCTTTCGATCCCACTTCTGATT 3'
LdhA (Right arm)	Sense: 5' CTTTAGGTTCTCAAGCTTATCACCTTGCGATCATCGACAT 3' Anti-sense: 5' CTTTAGGTTCTCACTAGTGTGGCAGCGCAAGTGTCCA 3'
Kn ^r gene set (Promoter + gene + terminator)	Sense: 5' CTTTAGGTTCTCGGTACCGGAAGCGGAACAGTAGAAAGC 3' Anti-sense: 5' GAAATGGTCTCCTCGAGGGTCATGATTCCGCGAACCCA 3'
LdhAp (−1042)	Sense: 5' CCGACAACACTGCACGGCCCTGCGA 3'
LdhAp (−202)	Sense: 5' CAATTCTGCAGGGCATAGGTTGG 3'
LdhA (+332)	Anti-sense: 5' CGCAGAGGAGAGGCGTTGCTGCAGG 3'
Promoter sequence	Amp^r promoter: 5' CGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACA TTCAAATATGTATCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT 3' PlacM promoter: 5' TGAGCTGTTTACAATTAATCATCGTGTGTGATCATGTGGAATTGGAAGGACTTGAACG 3' J23119 promoter: 5' TTATACCTAGGACTGAGCTAGCTGTCAA 3' Kn^r promoter: 5' GGAAGCGGAACACGTAGAAAGCCAGTCCGAGAAACGCTGCTGACCCCGGATGAATGTCAGC TACTGGGCTATCTGGACAAGGGAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGG CTTACATGGCGATAGCTAGACTGGGCGGTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGG GCGGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGGCCGCAAGAT CTGATGGCGCAGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCG 3' Psod promoter: 5' TGCGGAAACCTACGAAAGGATTTT 3'
Terminator sequence	rmB T1 terminator: 5' ATTTGTCCTACTCAGGAGAGCGTTACCGACAAACAACAGATAAAACGAAAGGCCAGTCTT TCGACTGAGCCTTTCGTTTTTATTT 3' SacB T1 terminator: 5' AAACGCAAAAGAAAATGCCGAT 3' Kn^r terminator 5' GCGGGACTCTGGGTTTCGCGGAATCATGACC3'
rpsL gene	5' ATGCCAACTATTACAGCAGTGGTCCGTAAGGGCCGCCACGATAAGTCCGCCAAGGTGGCTACC GCGGCACTGAAGGGTTCCCTCAGCGTCGTGGCGTATGCACCCGTGTGTACACCACCACCCCTAAG AAGCCTAACTCTGCTTTCGTAAGGTCGCTCGTGTGCGCCTTACCTCCGGCATCGAGGTTTCCGCTT ACATCCCTGGTGAGGGCCACAACCTGCAGGAGCACTCCATGGTGCTCGTTTCGCGGTGGTTCGTGTTA AGGACCTCCAGGTGTCGTTACAAGATCGTCCGTGGCGCACTGGATACCCAGGGTGTTAAGGACC GCAAGCAGGCTCGTTCCCG CTACGGCGCGAAGAGGGGATAA 3' (Under line: point mutagenesis site, AAG →AGG)
Kanamycin resistance gene target DNA	5' GAAATGGTCTCGGATCCGAATTTCTACTGTTGTAGATAACAAGATGGATTGCACGCAGGTTAA TTTAAATAAAACGAAAGGCTCAGTCG 3'
SucE	5' CTTTAGGTTCTCGAATTCATGAGCTTCTTTGTAGAAAATC 3' 5' GAAATGGTCTCAAGCTTGATAAGTAGGAACAACAACGTTTG 3'

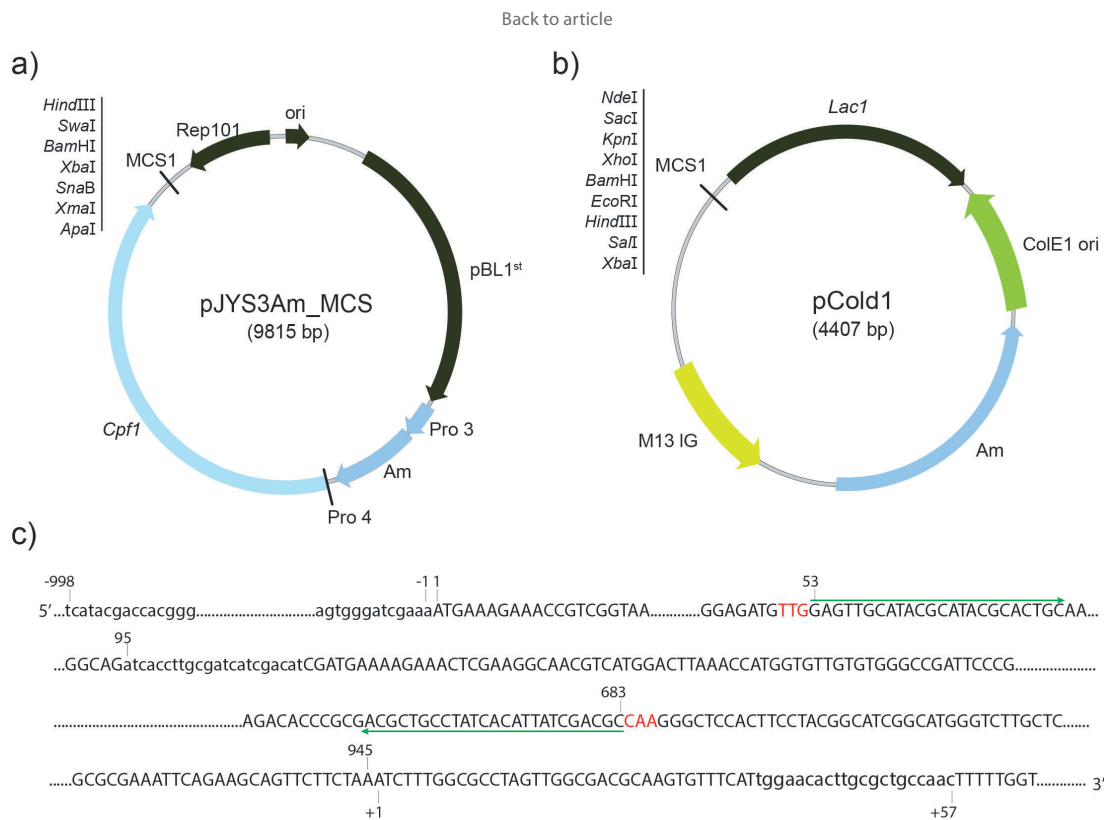


Fig. S1. The vectors used for generating CRISPR/Cpf1 system and for homologous recombination: a) the pJYS3_Am_MCS vector was derived from the pJYS3_ΔcrtYF plasmid, which was obtained from Addgene (www.addgene.org), b) the pCold vector (TaKaRa) was used as an intermediate vector to construct homologous arms and the expression gene set for the pJYS3Am_ *ldhA*-dT [*ldhA*_{pro}-Kn'-*ldhA*_{gen}] and pJYS3Am-Kn'-sT [*ldhA*_{pro}-Psod:*SucE*-rspLm-*ldhA*_{gen}] vectors, and c) the lactate dehydrogenase A (*ldhA*) gene and its flanking regions were used as homologous arms and two target DNA sequences for CRISPR/Cpf1 genome editing in *Corynebacterium glutamicum*. Lowercase letters indicate the primer annealing sites for PCR amplification of the promoter region (-1 to -998) and the region of the *ldhA* gene (95 to 945) along with a partial terminator region (+1 to +57). Capital letters denote the *ldhA* gene. Red letters represent PAM sites and green arrows indicate the target DNA sequences

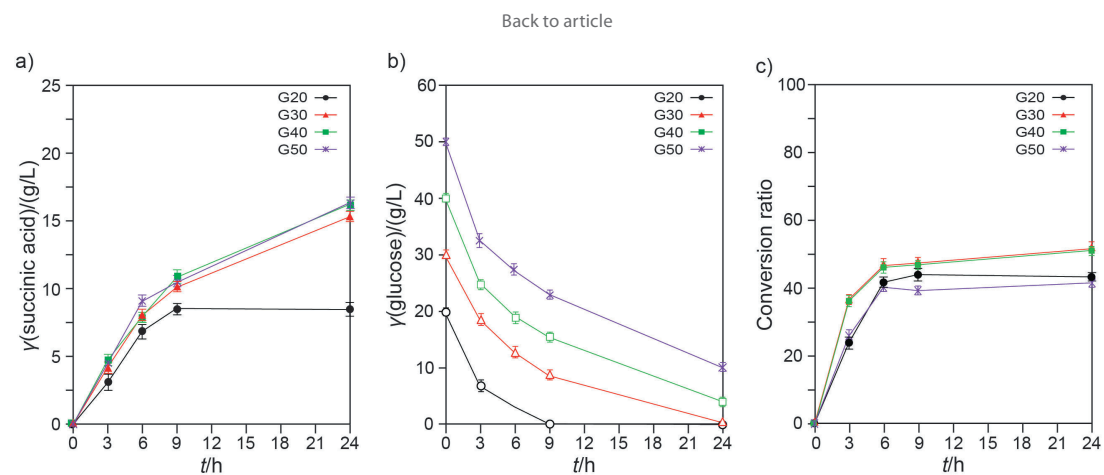


Fig. S2. Succinic acid production depends on glucose concentration: a) succinic acid production, b) glucose consumption, and c) the conversion ratio. G20, 30, 40 and 50=20, 30, 40 and 50 g/L of glucose