

Supplementary files

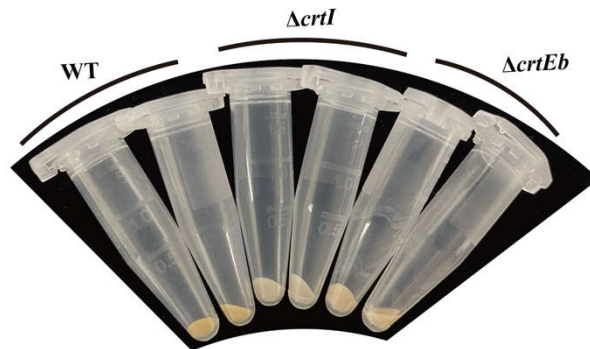


Figure S1 Colour comparison of the WT strain, $\Delta crtI$ strain, and $\Delta crtEb$ strain. From left to right strains are WT (*C. glutamicum* ATCC 13032, light yellow), $\Delta crtI$ (*C. glutamicum* ATCC 13032- $\Delta crtI$, white), and $\Delta crtEb$ (*C. glutamicum* ATCC 13032- $\Delta crtEb$, light pink).

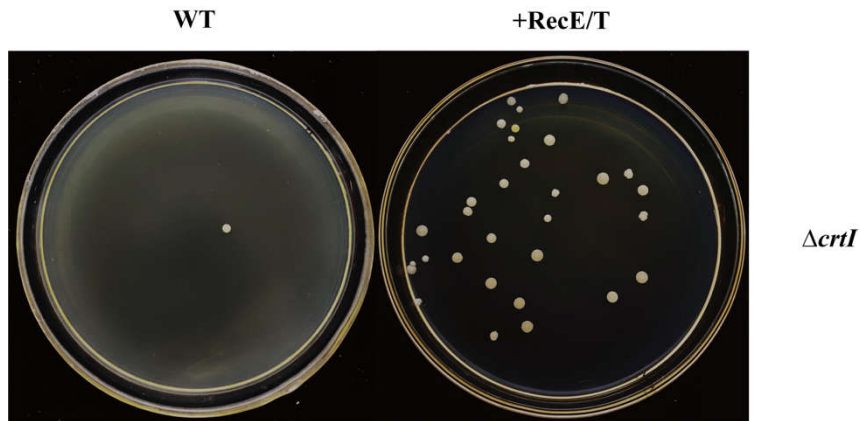


Figure S2 Effect of introduction of *recE/T* on the number of transformants. Both sides of the plate were introduced CRISPR/MAD7 system for $\Delta crtI$ in *C. glutamicum* ATCC 13032. The right plate was introduced *RecE/T* to promote homologous recombination, while the left did not.

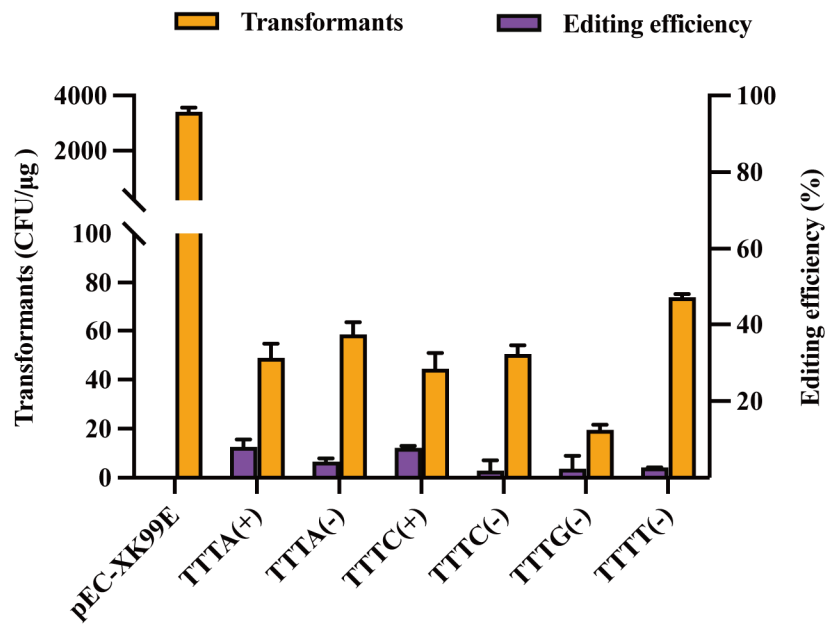


Figure S3 Transformants and editing efficiency of CRISPR/MAD7 using different PAM sequences. The effect of different PAM sequences on the transformants and the editing efficiency of $\Delta crtI$ by CRISPR/MAD7 system based on pEC-XK99E plasmid and *P_{trc}*-MAD7 in *C. glutamicum* ATCC 13032.

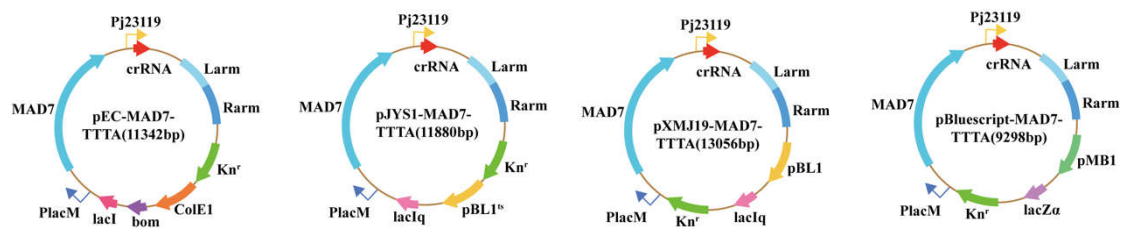


Figure S4 Schematic representation of the different plasmids carrying CRISPR/MAD7 system. All in one CRISPR/MAD7 system constructed using different plasmids, from left to right are based on pEC-XK99E, pJYS1Peftu, pXMJ19 and pBluescript respectively.

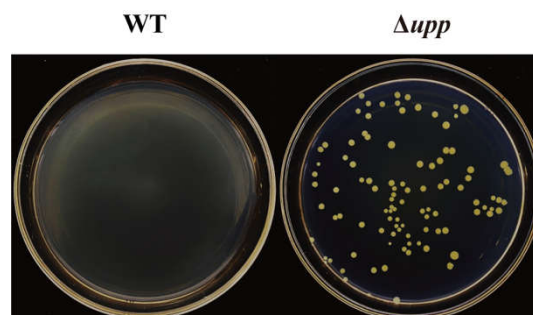


Figure S5 Comparison of the transformants number with knockout of the *upp* gene. The WT (*C. glutamicum* ATCC 13032) and Δupp (*C. glutamicum* ATCC 13032- Δupp) strain on the plates. Both the left and right plate is supplemented with 100 μ M 5-fluorouracil. Under normal circumstances, the colonies on the right plate will all be positive.

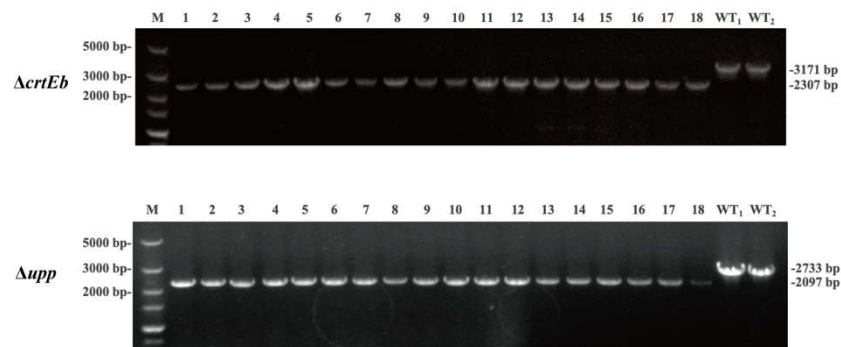


Figure S6 PCR validation of *crtEb* and *upp* gene deletion. From top to bottom, utilizing colony PCR for identification of *crtEb* and *upp* deletion in the genome of *C. glutamicum* ATCC 13032.

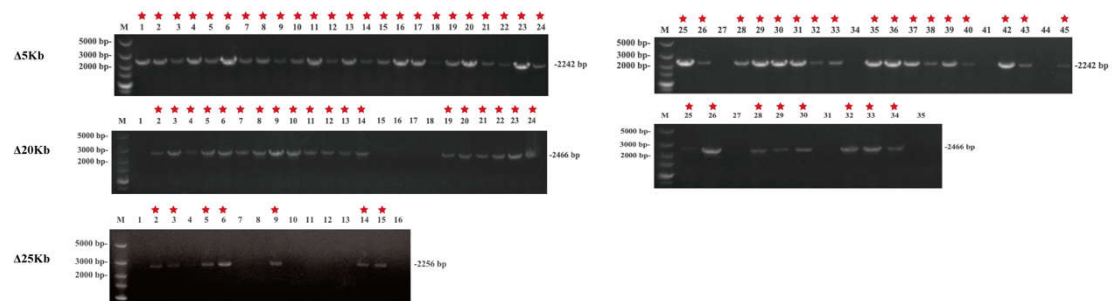


Figure S7 PCR validation of large DNA fragments deletion. From top to bottom, using colony PCR for identification of 5 kb, 20 kb, 25 kb DNA fragments deletion in the genome of *C. glutamicum* ATCC 13032.

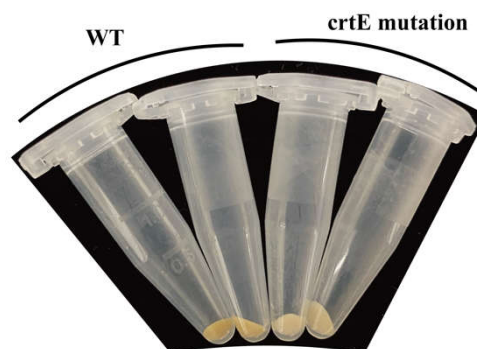


Figure S8 Colour comparison of the WT strain and *crtE* mutation strain. From left to right strains are WT (*C. glutamicum* ATCC 13032, light yellow), *crtE* mutation (*C. glutamicum* ATCC 13032-*crtE* mutation, white).

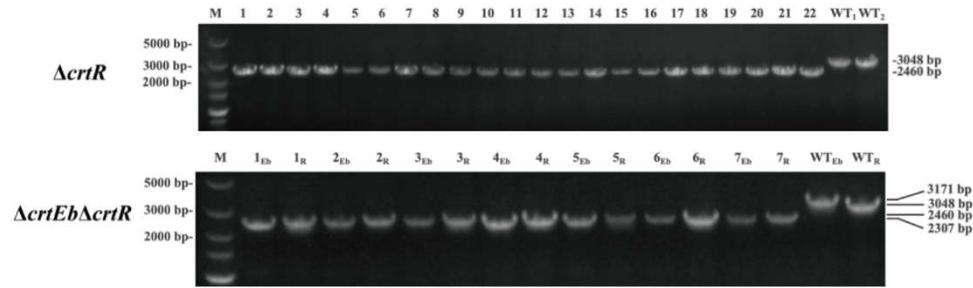


Figure S9 PCR validation of *crtR* gene and double gene deletion. From top to bottom, using colony PCR for identification of $\Delta crtR$, $\Delta crtEb\Delta crtR$ in *C. glutamicum* ATCC 13032.

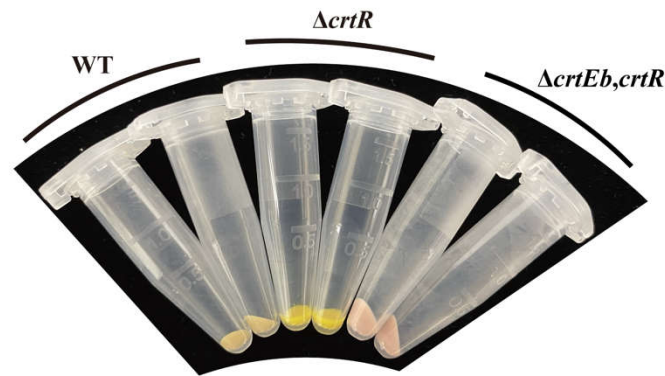


Figure S10 Colour comparison of the WT strain, $\Delta crtR$ strain, and $\Delta crtEb\Delta crtR$ strain. From left to right strains are WT (*C. glutamicum* ATCC 13032, light yellow), $\Delta crtR$ (*C. glutamicum* ATCC 13032- $\Delta crtR$, dark yellow), $\Delta crtEb\Delta crtR$ (*C. glutamicum* ATCC 13032- $\Delta crtEb\Delta crtR$, pink).

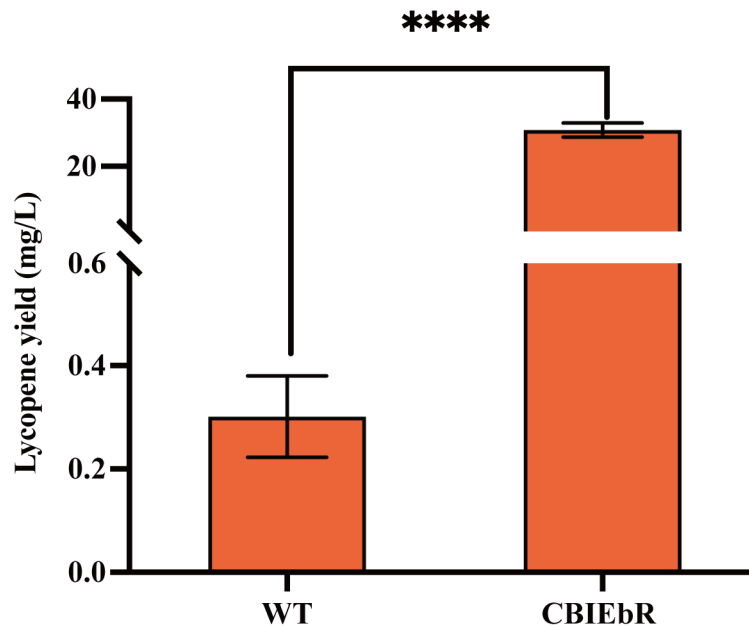


Figure S11 Lycopene production of WT strain and CBIEbR strain. Lycopene yield of WT strain and CBIEbR strain using CGXII medium (40 g/L glucose was added). Data are analyzed using two-tailed t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Table S1 Strains and plasmids used in this study.

Strains	properties	Source
<i>C. glutamicum</i> ATCC 13032	wild type	Buy from ATCC
<i>C. glutamicum</i> WT-RecE/T	ATCC 13032 contains pEC-RecE/T plasmid	This study
<i>C. glutamicum</i> WT-RecT	ATCC 13032 contains pEC-recT plasmid	This study
<i>E. coli</i> Top10F'	F' {lacIq Tn10 (Tet ^R)} <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) Φ80 <i>lacZ</i> Δ <i>M15</i> Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ (<i>ara-leu</i>) 7697 <i>galU</i> <i>galK</i> <i>rpsL</i> <i>endA1</i> <i>nupG</i>	Buy from ATCC
Plasmids		
pEC-XK99E	Kn ^r , pMB1 <i>ori</i> , <i>bom</i> , <i>lacI</i> , P _{trc} , <i>rrnB</i> T1, <i>rrnB</i> T2	Stock in the laboratory
pZ9	Kn ^r , p15A <i>ori</i> , <i>rep</i> , P _{tac} , <i>rrnB</i> T1, <i>rrnB</i> T2	Stock in the laboratory
pJYS1Peftu	Kn ^r , pBL1 ^{ts} <i>ori</i> , pSC101 <i>ori</i> , <i>lacIq</i> , P _{lacM-Fn} Cpf1, Peftu- <i>RecT</i>	Addgene
pXMJ19	Cm ^r , P _{lacIq} , <i>lacIq</i> , pBL1 <i>ori</i> , pUC <i>ori</i> , <i>rrnB</i> term	Addgene
pBluescript	Amp ^r , Cole1 <i>ori</i> , f1 <i>ori</i> , <i>lacZα</i>	Addgene
pEC-RecE/T	Cm ^r , pEC-RecE/T derivative for IPTG induced expression of RecE and RecT from Rac prophage from <i>E. coli</i>	Stock in the laboratory
pEC-recT	Cm ^r pEC-recT constructed by removing RecE from pEC-RecE/T	This study
pEC-MAD7	Kn ^r , pEC-XK99E carrying P _{trc} -MAD7	This study
pEC-MAD7-crRNA-TTTA (+)	Kn ^r , pEC-XK99E carrying P _{trc} -MAD7 and Pj23119-crRNA to target <i>crtI</i> gene, but without carrying homologous arm	This study
pEC-MAD7-TTTA (+)	Kn ^r , pEC-XK99E carrying P _{trc} -MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-MAD7-TTTA (-)	Kn ^r , pEC-XK99E carrying P _{trc} -MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-MAD7-TTTC (+)	Kn ^r , pEC-XK99E carrying P _{trc} -MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study

pEC-MAD7-TTTC (-)	Kn ^r , pEC-XK99E carrying Ptrc-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-MAD7-TTTG (-)	Kn ^r , pEC-XK99E carrying Ptrc-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-MAD7-TTTT (-)	Kn ^r , pEC-XK99E carrying Ptrc-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-Ptac-MAD7-TTTA (+)	Kn ^r , pEC-XK99E carrying Ptac-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-Ptuf-MAD7-TTTA (+)	Kn ^r , pEC-XK99E carrying Ptuf-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pEC-PlacM-MAD7-TTTA (+)	Kn ^r , pEC-XK99E carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pXMJ19-MAD7-TTTA (+)	Kn ^r , pXMJ19 carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pBluescript-MAD7-TTTA (+)	Kn ^r , pBluescript carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTA (+)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTA (-)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTC (+)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTC (-)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTG (-)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTT (-)	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study

pJYS1-MAD7-CTTT	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-CTTG	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-CTTA	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-CTTC	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtI</i> gene	This study
pJYS1-MAD7-TTTC- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-TTTG- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-CTTT- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-CTTG- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-CTTA- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-CTTC- <i>ΔcrtEb</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtEb</i> gene	This study
pJYS1-MAD7-TTTC- <i>Δupp</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study
pJYS1-MAD7-TTTG- <i>Δupp</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study
pJYS1-MAD7-CTTT- <i>Δupp</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study
pJYS1-MAD7-CTTG- <i>Δupp</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study

pJYS1-MAD7-CTTA- Δupp	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study
pJYS1-MAD7-CTTC- Δupp	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>upp</i> gene	This study
pJYS1-MAD7-TTTC- $\Delta 5$ kb	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target 5 kb gene	This study
pJYS1-MAD7-TTTC- $\Delta 20$ kb	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target 20 kb gene	This study
pJYS1-Cpf1-TTTC- $\Delta 20$ kb	Kn ^r , pJYS1Peftu carrying PlacM-Cpf1, Pj23119-crRNA, and homologous arm to target 20 kb gene	This study
pJYS1-MAD7-TTTC- $\Delta 25$ kb	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target 25 kb gene	This study
pJYS1-MAD7-TTTC- <i>crtE</i>	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtE</i> gene	This study
pJYS1-MAD7-TTTC- $\Delta crtR$	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA, and homologous arm to target <i>crtR</i> gene	This study
pJYS1-MAD7-TTTC- $\Delta crtEb\Delta crtR$	Kn ^r , pJYS1Peftu carrying PlacM-MAD7, Pj23119-crRNA array, and homologous arm to target <i>crtEb</i> and <i>crtR</i> gene	This study
pZ9- <i>crtE-crtB-crtI</i>	Kn ^r , pZ9 carrying one copy of the <i>crtE-crtB-crtI</i> gene	This study
pZ9- <i>cg0722-crtB-crtI</i>	Kn ^r , pZ9 carrying one copy of the <i>cg0722-crtB-crtI</i>	This study
pZ9- <i>crtE-cg0722-crtB-crtI</i>	Kn ^r , pZ9 carrying one copy of the <i>crtE-cg0722-crtB-crtI</i>	This study
pZ9- <i>idsA-idi-crtB-crtI</i>	Kn ^r , pZ9 carrying one copy of the <i>idsA-idi-crtB-crtI</i>	This study

Table S2 Primers used in this study.

Primers	DNA Sequence (5'to3')	Reference
P1	GAGAGAAGATTTTCAGCCTGATACAGAT	To amplify pEC-XK99E backbone fragment
P2	TCTGTTTCCTGTGTGAAATTGTTATCCGCT CACAATTC	

P3	TTTCACACAGGAAACAGAATATGAACAA CGGC	To clone pEC-MAD7
P4	CAGGCTGAAAATCTTCTCTCT	
P5	ATTAATGCAGCTGGCACGACA	To identify pEC-MAD7
P6	TAGGTGGAGGTATCGGAGAAA	
P7	TCCGATACCTCCACCTACGAA	
P8	CCGCTCGAGGGATGAATGTCAGCTACTGG G	To amplify crRNA-TTTA (+) fragment
P9	TCCCCGCGGCGATCCTCATCCTGTCTCTTG	
P10	CGAAGCGGCACCTTTAGTGCAT	To identify pEC-MAD7- crRNA-TTTA (+)
P11	AAGGCTCAGTCGAAAGACTGGGCCTTTTCG TTTTATGATGTTATACGGGATAAGAAA	To amplify $\Delta crtI$ homology arm
P12	AGCGCTCAAATAAAAACGAAAGGCTCAGT CG	
P13	ATCGATGATAGGGATCAAATTCACGAAT TCTTTCTGTAGATGCT	
P14	ATTTTGATCCCTATCATCGAT	
P15	GGTCGCGATGCCTGCTTTGTTCTACGACAT C	
P16	GTTTTTAAATCGACGGCGGCATCGAGTGC GTC	To clone pEC-MAD7-TTTA (+)
P17	CGTTTTATTTGAGCGCTAGTGCTAAAAGTG CAGAAGTG	
P18	CCCAGGAGGATTAATACCGCTAGCGCTCA AATAAAAACGAAAGGCTCAGTCG	To clone pEC-MAD7-TTTA (-)
P19	GGTATTAATCCTCCTGGGCGATCTACAAG AGTAGAAATTAAAAAGGTCTTTTGACACT	
P20	TTTAATTTCTACTCTTG TAGATGATGGGAT ACCGGACCTTCTTCTAGCGCTCAAATAA	To clone pEC-MAD7-TTTC (+)
P21	AGCGCTAGAAGAAGGTCCGGTATCCCATC ATCTACAAGAGTAGAAATTAAAAAGGTCT T	
P22	AGCGCTAGGTATCCAGCAGGTGCAACGCC ATCTACAAGAGTAGAAATTAAAAAGGTCT T	To clone pEC-MAD7-TTTC (-)
P23	TTTAATTTCTACTCTTG TAGATGGCGTTGC ACCTGCTGGATACCTAGCGCTCAAATAA	

P24	TTTAATTTCTACTCTTGTAGATCACTTTTCT GTTGTGAAGCAACTAGCGCTCAAATAA	To clone pEC-MAD7-TTTG (-)
P25	AGCGCTAGTTGCTTCACAACAGAAAAGTG ATCTACAAGAGTAGAAATTA AAAAAGGTCT T	
P26	TTTAATTTCTACTCTTGTAGATAGGCGGCA ATGGTTCGGGCAGCTAGCGCTCAAATAA	To clone pEC-MAD7-TTTT (-)
P27	AGCGCTAGCTGCCCCGAACCAT TGCCGCCT ATCTACAAGAGTAGAAATTA AAAAAGGTCT T	
P28	GCTAGCTCAGTCCTAGGTATAAT	To identify pEC-MAD7-TTTN (+)
P29	CATCGGCTCGTATAATGATGAACAACGGC ACCAACAAC	To clone pEC-Ptac-MAD7-TTTA (+)
P30	CATTATACGAGCCGATGATTAATTGTCAA CAGCTCATTT CAGAATATTTGCCAG	
P31	GATATCCTTCAGGATCTGGCG	To identify pEC-Ptac-MAD7-TTTA (+)
P32	GTCCAGGAGGACATACAATGAACAACGG CACCAACAAC	To clone pEC-Ptuf-MAD7-TTTA (+)
P33	TGTATGTCCTCCTGGACTTCGCAGCTCATT TCAGAATATTTGCCAG	
P34	TCTTTGATGATGCCGTTCTTCACGATG	To identify pEC-Ptuf-MAD7-TTTA (+)
P35	GTGTGGTACCATGTGTGGAATTGAAAGG ACTTGAACGATGAACAACGGCACCAACA AC	To clone pEC-PlacM-MAD7-TTTA (+)
P36	ACACATGGTACCACACGATGATTAATTGT AAACAGCCAGCTCATTT CAGAATATTTGC C	
P37	TCCAATCGATATCATCGATGGAG	To identify pEC-PlacM-MAD7-TTTA (+)
P38	GGTGCCGTTGTTTCATCGTTCAAGTCCTTTC CAATT	To clone pJYS1-MAD7-TTTA (+)
P39	CTAGGACTGAGCTAGCTGTCAACAGCCTG GCGGTGTAATGCA	
P40	GCTAGCTCAGTCCTAGGTATAAT	
P41	ATGAACAACGGCACCAACAA	
P42	GCAAAAACAACGAACCACACT	To identify pJYS1-MAD7-TTTA (+)
P43	GACTGAGCTAGCTGTCAACGAAATGACCG ACCAAGCGAC	To clone pXMJ19-MAD7-TTTA (+)

P44	TTTCTCTTTGCGCTTGCGGCCAGGGTGGTT TTTCTTTTCACCAGT	
P45	CGCAAGCGCAAAGAGAAAGCAG	
P46	TTGACAGCTAGCTCAGTCCTAGG	
P47	AACCCCGTTCGATATTTTGTG	To identify pXMJ19-MAD7-TTTA (+)
P48	TTTCTCTTTGCGCTTGCGCACATTTCCCCG AAAAGTGC	To clone pBluescript-MAD7-TTTA (+)
P49	GACTGAGCTAGCTGTCAACTGTCAGACCA AGTTTACTCATATATAC	
P50	GTAACAGGATTAGCAGAGCGA	To identify pBluescript-MAD7-TTTA (+)
P51	GTAGAACAAAGCAGGCAAGAATTTAAAT AAAACGAAAGGCTCAGTCG	To clone pJYS1-MAD7-TTTA (-) / TTTC (+) / TTTC (-) / TTTT (-) / TTTG (-)
P52	CTAGGACTGAGCTAGCTGTCAACAGCCTG GCGGTGTAATGCACC	
P53	ATGGGATACCGGACCTTCTCTAGCGCTCA AATAAAACGAAAGGC	To clone pJYS1-MAD7-CTTT
P54	AGGTCCGGTATCCCATCGATCTACAAGAG TAGAAATTAAAAAGGTCTTTTGACACTAG	
P55	AGCCATCACGTGCTAAAAGCTAGCGCTCA AATAAAACGAAAGGC	To clone pJYS1-MAD7-CTTG
P56	TTAGCACGTGATGGCTGGATCTACAAGAG TAGAAATTAAAAAGGTCTTTTGACACTAG	
P57	GATGCATTGTGGGGGCGGGATCTACAAGA GTAGAAATTAAAAAGGTCTTTTGACACTA G	To clone pJYS1-MAD7-CTTA
P58	CCCCACAATGCATCAACTAGCGCTCAAA TAAAACGAAAGGC	
P59	CTGTCTTCCCGACCCACTACTCTAGCGCTC AAATAAAACGAAAGGC	To clone pJYS1-MAD7-CTTC
P60	TGGGTCGGGAAGACAGATCTACAAGAGT AGAAATTAAAAAGGTCTTTTGACACTAG	
P61	TACGGAAGGATCTGAGGTTCT	To identify pJYS1-MAD7-YTTN
P62	AAAGGCTCAGTCGAAAGACTGGGCCTTTC GTTTTATGGGTGTAGGAATACCCATGTGTT	To amplify homology arm and crRNA for <i>ΔcrtEb</i>
P63	GAGGACTACGGCTTTTCTGGCTCA	
P64	CCAGAAAAGCCGTAGTCCTCTGCTTTTAT CCGATCAGTTCTG	

P65	TAAGCAAGACGCCCAATTTTCG	
P66	TGCTGAATAAGCAATCACTCTAGCGCTCA AATAAAACGAAAGGCTCAGTCGAAAGAC TG	
P67	TGGGCGTCTTGCTTAAGAATTTAAATAAA ACGAAAGGCTCAGTCG	To clone pJYS1-MAD7-TTTC- <i>ΔcrtEb</i>
P68	GAGTGATTGCTTATTCAGCACCATCTACA AGAGTAGAAATTA AAAAAGGTCTTTTGACA C	
P69	CATTACAGATGCCAGTTGCTAGCGCTCAA ATAAAACGAAAGGCTCAGTCGAAAGACT G	To clone pJYS1-MAD7-TTTG- <i>ΔcrtEb</i>
P70	CAACTGGCATCTGTAATGTTTCATCTACAA GAGTAGAAATTA AAAAAGGTCTTTTGACAC	
P71	TGACACTCTCAGTGCTCTAGCGCTCAAAT AAAACGAAAG	To clone pJYS1-MAD7-CTTT- <i>ΔcrtEb</i>
P72	AGCACTGAGAGTGTGAGCCATATCTACAA GAGTAGAAATTA AAAAAGGTCTTTTGACAC	
P73	GCAGTACAGGATGTTAATGCTAGCGCTCA AATAAAACGAAAGGCTCAGTCGAAAGAC TG	To clone pJYS1-MAD7-CTTG- <i>ΔcrtEb</i>
P74	CATTAACATCCTGTACTGCTCATCTACAA GAGTAGAAATTA AAAAAGGTCTTTTGACAC	
P75	CGTATAACATCGCCATGTCTAGCGCTCAA ATAAAACGAAAGGCTCAGTCGAAAGACT G	To clone pJYS1-MAD7-CTTA- <i>ΔcrtEb</i>
P76	ACATGGCGATGTTATACGGGAATCTACAA GAGTAGAAATTA AAAAAGGTCTTTTGACAC	
P77	GTCTGCATTAACATCCTGCTAGCGCTCAA ATAAAACGAAAGGCTCAGTCGAAAGACT G	To clone pJYS1-MAD7-CTTC- <i>ΔcrtEb</i>
P78	CAGGATGTTAATGCAGACCGGATCTACAA GAGTAGAAATTA AAAAAGGTCTTTTGACAC	
P79	AAGTCATGGTTCAACCTCGG	To identify pJYS1-MAD7- YTTN- <i>ΔcrtEb</i>
P80	TGCAGCAGCCAACGACCTCCTAGCGCTCA AATAAAACGAAAGGCTCAGTCGAAAGAC TG	To amplify homology arm and crRNA for <i>Δupp</i>
P81	CGAAAGGCTCAGTCGAAAGACTGGGCCTT TCGTTTTATAAGCCCACTGTGTAAACCGC	
P82	TGCCCTTAGAACTTAGCTTCACATGTTAA ATCATTGCCG	

P83	GAAGCTAAGTTTCTAAGGGCATTACGGAA AATTCTTGCCC	
P84	CCGAGACACTGACGGGATTCA	
P85	CCGTCAGTGTCTCGGAGAATTTAAATAAA ACGAAAGGCTCAGTCGAAAGAC	To clone pJYS1-MAD7-TTTC- <i>Δupp</i>
P86	TCGTTGGCTGCTGCACGATCTACAAGAGT AGAAATTAAAAAGGTCTTTTGACAC	
P87	TCGATGGTGGCGGTAACATCTACAAGAGT AGAAATTAAAAAGGTCTTTTGACAC	To clone pJYS1-MAD7-TTTG- <i>Δupp</i>
P88	TACCGCCACCATCGACCCACTAGCGCTCA AATAAAACGAAAGGCTCAGTCGAAAGAC TG	
P89	TCGTTGGCTGCTGCACGGATCTACAAGAG TAGAAATTAAAAAGGTCTTTTGACAC	To clone pJYS1-MAD7-CTTT- <i>Δupp</i>
P90	TGCAGCAGCCAACGACCTCTAGCGCTCAA ATAAAACGAAAGGCTCAGTCGAAAGACT G	
P91	CCGGTGATCGTCTCTCTAGCGCTCAAATA AAACGAAAGGCTCAGTCGAAAGACTG	To clone pJYS1-MAD7-CTTG- <i>Δupp</i>
P92	AGAGACGATCACCGGCATCTCATCTACAA GAGTAGAAATTAAAAAGGTCTTTTGACAC	
P93	TGGCAGCGCCTCAACTAGCGCTCAAATAA AACGAAAGGCTCAGTCGAAAGACTG	To clone pJYS1-MAD7-CTTA- <i>Δupp</i>
P94	TTGAGGCGCTGCCACAGGATCATCTACAA GAGTAGAAATTAAAAAGGTCTTTTGACAC	
P95	AGTACCTTCAGCCATGCTAGCGCTCAAAT AAAACGAAAGGCTCAGTCGAAAGACTG	To clone pJYS1-MAD7-CTTC- <i>Δupp</i>
P96	CATGGCTGAAGGTACTCGCCTATCTACAA GAGTAGAAATTAAAAAGGTCTTTTGACAC	
P97	CAACAACACCACCTCCTTGT	To identify pJYS1-MAD7- YTTN- <i>Δupp</i>
P98	AGGCTCAGTCGAAAGACTGGGCCTTTCGT TTTATGTCTCAATTTCTGGATTGAGCCTG	To amplify Δ5 kb homology arm and crRNA
P99	ACTCGCGGGCAATAACGTAGAGCGATAG TCTGCGGG	
P100	TTATTGCCCGCGAGTCTGCTG	
P101	GATTTGCTTTGCGGTATCCCAAC	
P102	CACCCATGACCTCTCTAGCGCTCAAATAA AACGAAAGGCTCAGTCGAAAGACTGGG	To clone pJYS1-MAD7-TTTC- Δ5 kb
P103	TACCGCAAAGCAAATCAGAATTTAAATA AAACGAAAGGCTCAGTCG	

P104	AGAGGTCATGGGTGTGTAGTAATCTACAA GAGTAGAAATTAAAAAGGTCTTTTGACAC	
P105	CAACTCACCGACAATGCCGAAA	To identify pJYS1-MAD7- TTTC-Δ5 kb
P106	CGGTCTGAATGGTGTTCATCGG	
P107	TGGTCATGCACCACATTGATGAGCAC	To amplify Δ 20kb homology arm
P108	TTCGCCGATGCCGTAGATGAATGAGGTTT TACCG	
P109	ATCTACGGCATCGGCGAAGGTCAAGATCA GGGTTATTAACCATTTTCTGCGC	
P110	ATTCGTCTCACTGATACTCGCGCCAA	
P111	GGCTCAGTCGAAAGACTGGGCCTTTCGTT TTATTGGTCATGCACCACATTGATGAGCA C	To clone pJYS1-MAD7-TTTC- Δ20 kb
P112	CGCGAGTATCAGTGAGACGAATAGAATTT AAATAAAACGAAAGGCTCAGTCG	
P113	GAAGAAGTACACGTTCTTGGTGAGTGC	To identify pJYS1-MAD7- TTTC-Δ20 kb
P114	AATGACCAGATGCGACTGCCCAATA	
P115	AGAGGTCATGGGTGTGTAGTAATCTACAA GAGTAGAAATTAAAAAGGTCTTTTGACAC	To clone pJYS1-Cpf1-TTTC- Δ20 kb
P116	CGCGAGTATCAGTGAGACGAATAGAATTT AAATAAAACGAAAGGCTCAGTCG	
P117	TTAGCGGACTTGAAGTCCTTCTGCA	To identify pJYS1-Cpf1-TTTC- Δ20 kb
P118	CCGACAAGAAGTTCTTCGCAAAGCT	
P119	TCTCAGACATCAACAAACGCCCCA	To amplify Δ25 kb homology arm
P120	GCGGAATTGTGGGACACCGTATCTGTGTT GGTCTCTATTATGCCGGA	
P121	ATACGGTGTCCCACAATTCCGC	
P122	AAGCAATAGGATCTGTGTGCTGGT	
P123	CACACAGATCCTATTGCTTAGAATTTAAA TAAACGAAAGGCTCAGTCG	To clone pJYS1-MAD7-TTTC- Δ25 kb
P124	TTACTTCTTCAGCGATCTAGCGCTCAAATA AAACGAAAGGCTCAGTCGAAAGACTGGG C	
P125	GATAACTCACCGTTTGATCCGG	

P126	CTTTAATGATGGGGACATGCGC	To identify pJYS1-MAD7-TTTC- Δ 25 kb
P127	TAAGCAGTCCATTAGATTCTAGCGCTCAA ATAAAACGAAAGGCTCAGTCGAAAGACT GG	To clone pJYS1-MAD7-TTTC- <i>crtE</i>
P128	TCTAATGGACTGCTTATGGATCTACAAGA GTAGAAATTAAAAAGGTCTTTTGACACTA G	
P129	CTGAAGGGCCTCCTTTTTGTATCCG	To clone pEC-recT
P130	AAGGAGGCCCTTCAGATGACTAAGCAAC CACCAATCGCAAA	
P131	CTGTTTGCGCGTCATAACTTC	To identify pEC-recT
P132	ATGGACAATGGCATGACAATCAC	To amplify <i>crtE</i> sequence
P133	CTAAGATTTGCGGCTGGCTAGT	
P134	TGGGTGGCAGCAAGAAAAAGATTAACCA TAAGCAGTCCATTAGATTGTGCCCAATCT AG	59 bp, corresponding to the mutation of PAM (TTTC) on the <i>crtE</i> gene
P135	AAGGTCAAGGCGCGCGAAGATTTGATGG GTGGCAGCAAGAAAAAGATTAACCATAA GCAGTCCATTAGATTGTGCCCAATCTAGA TGCTCTGGATCGCG	100 bp, corresponding to the mutation of PAM (TTTC) on the <i>crtE</i> gene
P136	TCGGCTGCGTCACCAAAAGTA	To amplify Δ <i>crtR</i> homology arm
P137	CACTTTAATTAACGTAGATGGTCATAGTA ATCACCTTTTTCTACAAAGTCAGATAAAG	
P138	ATGACCATCTACGTTAATTAAGTGTGCA GAG	
P139	AGTCAAAGCGCTGAAGACTTGT	
P140	TACTTTTGGTGACGCAGCCGAATAAAACG AAAGGCCAGTCT	To clone pJYS1-MAD7-TTTC- Δ <i>crtR</i>
P141	ACAAGTCTTCAGCGCTTGACTAGAATTT AAATAAAACGAAAG	
P142	CAGCAGTGCGTTGCTCTTGCT	To identify pJYS1-MAD7-TTTC- Δ <i>crtR</i>
P143	CAGTATCTCGAGCGAAGGCGA	
P144	AGTGATTGCTTATTCAGCACC	To clone pJYS1-MAD7-TTTC- Δ <i>crtEb</i> Δ <i>crtR</i>
P145	GCTGAATAAGCAATCACTGTCAAAAGAC CTTTTAAATTCTACTCTTGATAGATCC	

P146	ACACATGGGTATTCCTACACCCATAAAAC GAAAGGCCCAGTCTTTC	
P147	CGAAATTGGGCGTCTTGCTTATCGGCTGC GTCACCAAAAGTA	
P148	TTAACCTTGCACCCCATATGC	
P149	GCGCCATATACAACGATTGGGAA	To identify pJYS1-MAD7- TTTC- $\Delta crtEb\Delta crtR$
P150	GAAAGGAGGCCCTTCAGATGGAC	To clone pZ9- <i>crtE-crtB-crtI</i>
P151	CTGAAGGGCCTCCTTTCCTAAGATTTGCG GCTGGCTAGT	
P152	CCAGCCGCAAATCTTAGGAAAGGAGGCC CTTCAGATGACACACCAAAATTCGCCTCT	
P153	AGTTCCTACTCTCGCATCCTTAATGATCG TATGAGGTC	
P154	GGATGCGAGAGTAGGGA ACT	
P155	CTGAAGGGCCTCCTTCTTGTTATCCGC	
P156	CTGCCGTTGATGATCAAAACA	
P157	TTACATGCCGATACCAGTGCAG	To identify pZ9- <i>crtE-crtB-crtI</i>
P158	GGTCTCCGTCAATAACATCGT	
P159	CCAGCCGCAAATCTTAGGAAAGGAGGCC CTTCAGATGTCTACTAGCATCACAAC	
P160	GAATTTTGGTGTGTCATCTGAAGGGCCTCC TTTCTCATAGCTGAGGCTGCTTCT	To clone pZ9- <i>cg0722-crtB-crtI</i> and pZ9- <i>crtE-cg0722-crtB-crtI</i>
P161	GAAAGGAGGCCCTTCAGATGACACACCA AAATTCGCCTCT	
P162	TTGCCTTCATTGTCGCTTTTG	
P163	ATTTTGGCAGTTGAGCAACCA	To identify pZ9- <i>cg0722-crtB-crtI</i>
P164	TGCCTGCTTTGTTCTACGACAT	To identify pZ9- <i>crtE-cg0722-crtB-crtI</i>
P165	GATATTCAAGACA ACTTCGGC	
P166	TTGTTATCCGCTCACAATTCC	
P167	TTGTGAGCGGATAACAAGAAAGGAGGCC CTTCAGATGAGCAGTTTCGATGCC	To clone pZ9- <i>idsA-idi-crtB-crtI</i>
P168	CTACATCCGACGTTTCGGTTG	

P169	CAACCGAACGTCGGATGTAGGAAAGGAG GCCCTTCAGATGACTACTGAGGTTGAACT GG	
P170	CTGAAGGGCCTCCTTTCTTACTCTGCGTCA AACGCTTCC	
P171	GAAATTTGTCGAGGTCAAGGT	To identify pZ9- <i>idsA-idi-crtB-crtI</i>
P172	GTGGAAAACGCGAAATGCAGAGG	
P173	CTTTTAAGGAAGAGAGGCGAAT	

Table S3 Editing efficiency of large DNA fragment deletion in *C. glutamicum*.

	crRNA	PAM	Efficiency
Δ5 kb	TACTACACACCCATGACCTCT	TTTC	15/15;14/15;12/15
Δ20 kb	TACTACACACCCATGACCTCT	TTTC	7/8;9/13;11/14
Δ25 kb	TACTACACACCCATGACCTCT	TTTC	2/4;3/9;2/3