

**CRISPR/Cas12a mediated genome editing to introduce  
amino acid substitutions into the mechanosensitive  
channel MscCG of *Corynebacterium glutamicum***

Karin Krumbach<sup>1</sup>, Christiane Katharina Sonntag<sup>1</sup>, Lothar Eggeling<sup>1</sup>,  
and Jan Marienhagen<sup>1,2,\*</sup>

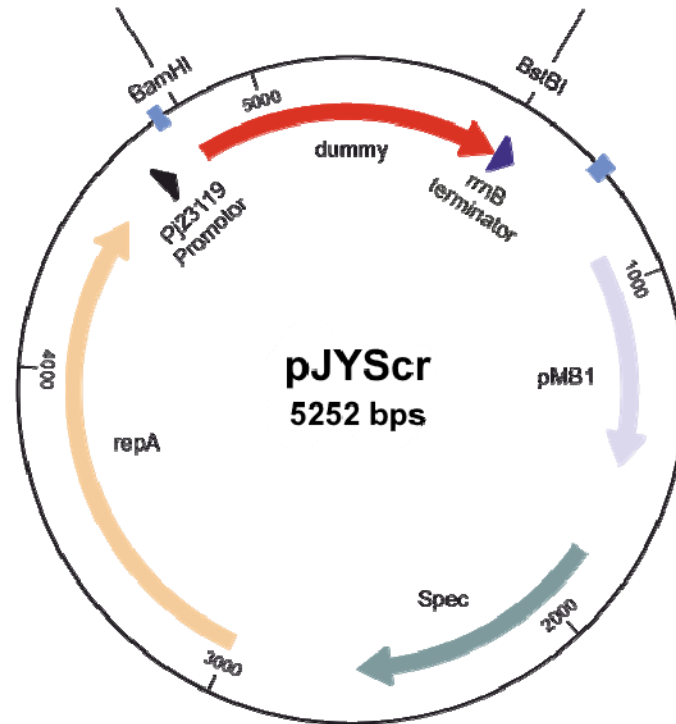
<sup>1</sup>Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, D-52425 Jülich, Germany

<sup>2</sup>Institute of Biotechnology, RWTH Aachen University, Worringer Weg 3, D-52074 Aachen, Germany

\* To whom correspondence should be addressed.

Tel: +49 2461 61 2843; Fax: +49 2461 61 2710; Email: [j.marienhagen@fz-juelich.de](mailto:j.marienhagen@fz-juelich.de)

5'-GATCCGAATTCTACTGTTGTAGATAGCACCTCAAGTCCAGCTGCCGTTT-3'  
GC TTAAGATGACAACATCTATCGTGGAGTTCAGGTGACGCAAGC



**Supplementary Figure S1:** Plasmid map of the pJYScr vector constructed in this study for easy incorporation of crRNA encoding oligonucleotides. The 931 *Bam*HI-*Bst*BI-“dummy” fragment with the relevant restrictions sites for easy subcloning is highlighted.

**Supplementary Table S1.** Plasmids and oligonucleotides used for plasmid constructions.

<b>Plasmids</b>	<b>Characteristics</b>	<b>Source</b>
pK19mobsacB	Integrative vector, Km <sup>r</sup> <i>mob sacB</i>	(1)
pEKEx3	Shuttle vector, Spc <sup>r</sup> <i>Ptac lac<sup>h</sup></i>	(2)
pJYS2_crtYf	Shuttle vector based on pMB1, Spc <sup>r</sup> , Pj23119-crRNA targeting <i>crtYf</i>	(3)
pJYS1	Shuttle vector based on pBL1, Km <sup>r</sup> , RecT, <i>FnCpf1</i> , identical with pJYS1-petFu	(3)
pMK-RQ_PH36_LacZ	Vector providing <i>lacZ</i> under constant high expression of PH36	invitrogen Thermo Fisher Scientific
pMA-RQ_crRNA_crtYf	Vector providing a 313 bp fragment in which a <i>DraI</i> site of pJYS2_crtYf was replaced by a <i>BstBI</i> site	invitrogen Thermo Fisher Scientific

<b>Oligonucleotides</b>	<b>Sequence (5'-3')</b>	<b>Purpose</b>
cr:lacZ 1574f_fw	CTGTTGTAGATGCTACCTGGAGAGACGC GCCCATTCGAAATAAAACGAAAGGCTC	pJYScr-lacZ-1574
cr:lacZ 1574f_rv	CGTTTTATTTTCGAATGGGCGCGTCTCTC CAGGTAGCATCTACAACAGTAGAAATTC G	pJYScr-lacZ-1574f
cr:lacZ1656c_fw	CTGTTGTAGATGCGAAACCGCCAAGACT GTTAATTCGAAATAAAACGAAAGGCTC	pJYScr-lacZ-1654c
cr:lacZ1656c_rev	CGTTTTATTTTCGAATTAACAGTCTTGGCG GTTTCGCATCTACAACAGTAGAAATTCG	pJYScr-lacZ-1654c
cr:murE-2_BamHI	GATCCGAATTTCTACTGTTGTAGATAGC ACCTCAAGTCCAGCTGCGTTT	pJYScr:murE
cr:murE-2_BstBI	CGAA ACGCAGCTGGACTTGAGGTGCTATCTAC AACAGTAGAAATTCG	pJYScr:murE
cr:mscCG_1269c_fw	GATCCGAATTTCTACTGTTGTAGATCAC AGTCATGACCTTAAATAGTTT	pJYScr_mscCG1269c
cr:mscCG_1269c_rv	CGAAACTATTTAAGGTCATGACTGTGAT CTACAACAGTAGAAATTCG	pJYScr_mscCG1269c
m420stopΔ-1fwd	tgcctgcctgcaggctgactGGCGGATCGACCAC GGCT	pK19mobsacB-420stopΔ
m420stopΔ-2rev	cagcgtcctaGACCTTAAATAGTGACAGCAA	pK19mobsacB-

	GAGCAGC	420stopΔ
m420stopΔ-3fwd	atttaaggtcTAGGACGCTGATTACAGAC	pK19mobsacB-420stopΔ
m420stopΔ-4rev	ttgtaaacgacggccagtgGTAGCCGTCTTCTT GAAC	pK19mobsacB-420stopΔ
p424stopΔ-1fwd	cctgcaggtcgactctagagCCGGCAATCAATGG CTGG	pK19mobsacB-424stopΔ
p424stopΔ-2rev	AATCAGCGTCTTATTATTCCACAGTCATG ACCTTAAATAGTG	pK19mobsacB-424stopΔ
p424stopΔ-3fwd	GGAATAATAAGACGCTGATTACAGACGT G	pK19mobsacB-424stopΔ
p424stopΔ-4rev	ttgtaaacgacggccagtgGTAGCCGTCTTCTT GAAC	pK19mobsacB-424stopΔ
pJYS2_fw2	GTGTCAGTGAAAGGCGCATCC	Sequencing primer
pJYS2_rv2	TCGCCACCTCTGACTTGAGCG	Sequencing primer

---

**Supplementary Table S2.** Recombinogenic oligos and obtained mutations at two *lacZ* locations. Shown on top is the *lacZ* sequence targeted for mutations using recombineering in combination with CRISPR/Cas12. The PAM sequence is underlined. Used recombinogenic oligonucleotides (o-1574x or o-1654x) have a grey background with the nucleotides deviating from the *lacZ* sequence in red. The DNA sequencing results for individual clones are given below the oligonucleotide sequences.

***lacZ***      CC ATC AAA AAA TGG CTT TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

**o-1574a**      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 1      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 2      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 3      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 4      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 5      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 6      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 7      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 8      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 8      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 9      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 10      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 11      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 12      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 13      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 14      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 15      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 16      CC ATC AAA AAA TGG CTA TAG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

**o-1574b**      CC ATC AAA TAG TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 1      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 2      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 3      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 4      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 5      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 6      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 7      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 8      CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

**o-1574c**      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 1      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 2      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 3      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 4      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 5      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 6      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 7      CC ATC AAA AAA TAG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC









**Supplementary Table S3.** Recombinogenic oligos for the insertion of a random stretch of nucleotides and codon saturation mutagenesis. On top always the *lacZ* sequence is shown, targeted by recombineering in combination with CRISPR/Cas12a. The PAM sequence is underlined. Used recombinogenic oligonucleotides have a grey background with the nucleotides deviating from the *lacZ* sequence in red. The DNA sequencing results for individual clones are given below the oligonucleotide sequences. With the oligonucleotide o-1574fe-random carrying a stretch of 18 random nucleotides, only few white colonies were obtained (see results section in the main text). This was also the case with an additional, independently synthesized oligonucleotide with identical sequence. Noteworthy, clones 7 and 8 obtained during experiments with this oligonucleotide appeared white, but do not carry any additional mutation near the PAM site. It is possible that these two clones carry additional unwanted mutations in the *lacZ* gene. However, the nucleotide sequence nt 1188 to nt 1902 of these two clones was not at variance from the wild type sequence. Results obtained during codon saturation mutagenesis (oligonucleotides o-1574e-mix and o-1574g-mix), the amino acid substitution obtained is indicated in addition to the nucleotide sequence.

***lacZ*** CC ATC AAA AAA TGG CTT TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

o-lacZ\_1574fe-random CC ATC AAA AAA TCC GAA AGC GAT GGA TAG GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 1	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG TAA CGG CCG CTG ATC CTT TGC GAA TAC
clone 2	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA TAG ACG CGG CCG CTG ATC CTT TGC GAA TAC
clone 3	CC ATC AAA AAA TGG TAG TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC
clone 4	CC ATC AAA AAA TGG TAA TCG CTA CCT GGA AAG ACA CGG CCG CTG ATC CTT TGC GAA TAC
clone 5	CC ATC AAA AAA TGG TAG TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC
clone 6	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA TCG ACG CGG CCG CTG ATC CTT TGC GAA TAC
clone 7	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC
clone 8	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

o-1574e-mix CC ATC AAA AAA TGG CTA TCG NNN CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC

clone 1	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 2	CC ATC AAA AAA TGG CTA TCG GCC CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527A
clone 3	CC ATC AAA AAA TGG CTA TCG AGA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527R
clone 4	CC ATC AAA AAA TGG CTA TCG AAC CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527N
clone 5	CC ATC AAA AAA TGG CTA TCG TAC CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527Y
clone 6	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 7	CC ATC AAA AAA TGG CTA TCG TCT CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527S
clone 8	CC ATC AAA AAA TGG CTA TCG ATT CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527I
clone 9	CC ATC AAA AAA TGG CTA TCG TAA CCT GGA --- ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527stop
clone 10	CC ATC AAA AAA TGG CTA TCG ACC CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527T
clone 11	CC ATC AAA AAA TGG CTA TCG TCT CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527S
clone 12	CC ATC AAA AAA TGG CTA TCG GGG CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527G
clone 13	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 14	CC ATC AAA AAA TGG CTA TCG TGT CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527C
clone 15	CC ATC AAA AAA TGG CTA TCG CAG CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527Q
clone 16	CC ATC AAA AAA TGG C-A TCG GCG CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 17	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 18	CC ATC AAA AAA TGG CTA TCG GCG CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527A

amino acid

clone 19	CC ATC AAA AAA TGG CTA TCG ATC CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527I
clone 20	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 21	CC ATC AAA AAA TGG CTT TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 22	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 23	CC ATC AAA AAA TGG CTA TCG TAT CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	L527Y
clone 24	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTGG ATC CTT TGC GAA TAC	

o-1574g-mix	CC ATC AAA AAA TGG CTA TCG CTA CCT NNN GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 1	CC ATC AAA AAA TGG CTA TCG CTA CCT GGC GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529G
clone 2	CC ATC AAA AAA TGG CTA TCG CTA CCT AAA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529K
clone 3	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 4	CC ATC AAA AAA TGG CTA TCG CTA CCT AGC GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529S
clone 5	CC ATC AAA AAA TGG CTA TCG CTA CCT GAA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529E
clone 6	CC ATC AAA AAA TGG CTA TCG CTA CCT TAC GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529Y
clone 7	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 8	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 9	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 10	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 11	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 12	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 13	CC ATC AAA AAA TGG CTA TCG CTA CCT TTA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529L
clone 14	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 15	CC ATC AAA AAA TGG CTA TCG CTA CCT AGC GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529S
clone 16	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 17	CC ATC AAA AAA TGG CTA TCG CTA CCT TAG GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529stop
clone 18	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 19	CC ATC AAA AAA TGG CTA TCG CTA CCT GTA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529V
clone 20	CC ATC AAA AAA TGG CTA TCG CTA CCT GGA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	
clone 21	CC ATC AAA AAA TGG CTA TCG CTA CCT TTG GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529L
clone 22	CC ATC AAA AAA TGG CTA TCG CTA CCT TAA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529stop
clone 23	CC ATC AAA AAA TGG CTA TCG CTA CCT GAA GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529E
clone 24	CC ATC AAA AAA TGG CTA TCG CTA CCT TGC GAG ACG CGG CCG CTG ATC CTT TGC GAA TAC	G529C

**Supplementary Table S4.** Recombinogenic oligonucleotides used for introducing mutations in *mscCG* and the mutations obtained. Shown on top is the targeted *mscCG* sequence with the PAM sequence underlined (PAM sequence is on the opposite strand). The recombinogenic oligonucleotides o-*mscCG*1269-V422nnn and o-*mscCG*1xxx-E423 used are highlighted by a grey background. The DNA sequencing results for all individual clones with nucleotides deviating from the wild type sequence are presented in red. In the right column the relevant amino acid sequences (amino acids 420 - 426) are shown.

<i>mscCG</i>	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <u>GAA</u> CCA AGT GAG AAT TGG CAA	- Met Thr Val Glu Pro Ser Glu -
<b>o-<i>mscCG</i>1269-V422nnn</b>	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>nnn</b> GAG CCA AGT GAG AAT TGG CAA	
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>tTa</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Leu Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>GCT</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Ala Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>AAA</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Lys Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>TGC</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Cys Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>ACT</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Thr Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>CTC</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Leu Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>TTA</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Leu Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>GAT</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Asp Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>TCG</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Ser Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>ACT</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Thr Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>CTC</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Leu Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>ACA</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Thr Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>TGC</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Cys Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>ACT</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Thr Glu Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT <b>CTG</b> GAG CCA AGT GAG AAT TGG CAA	- Met Thr Leu Glu Pro Ser Glu -
<i>mscCG</i>	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <u>GAA</u> CCA AGT GAG AAT TGG CAA	- Met Thr Val Glu Pro Ser Glu -
<b>o-<i>mscCG</i>1269-E423nnn</b>	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <b>nnn</b> CCA AGT GAG AAT TGG CAA	
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <b>CCG</b> CCA AGT GAG AAT TGG CAA	- Met Thr Val Pro Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <b>AAC</b> CCA AGT GAG AAT TGG CAA	- Met Thr Val Asn Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <b>ACC</b> CCA AGT GAG AAT TGG CAA	- Met Thr Val Thr Pro Ser Glu -
	CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG <b>TCC</b> CCA AGT GAG AAT TGG CAA	- Met Thr Val Ser Pro Ser Glu -

CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GAC CCA AGT GAG AAT TGG CAA - Met Thr Val Asp Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG TAA CCA AGT GAG AAT TGG CAA - Met Thr Val STOP  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GAA CCA AGT GAG AAT TGG CAA - Met Thr Val Glu Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG TCA CCA AGT GAG AAT TGG CAA - Met Thr Val Ser Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GT- -AG CTC AGT GAG AAT TGG TGA - Met Thr Val Ala Gln STOP  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG ACA CCA AGT GAG AAT TGG CAA - Met Thr Val Thr Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GCA CCA AGT GAG AAT TGG CAA - Met Thr Val Ala Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GAC CCA AGT GAG AAT TGG CAA - Met Thr Val Asp Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG AAC CCA AGT GAG AAT TGG CAA - Met Thr Val Asn Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG AAA CCA AGT GAG AAT TGG CAA - Met Thr Val Lys Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GCC CCA AGT GAG AAT TGG CAA - Met Thr Val Ala Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GCC CCA AGT GAG AAT TGG CAA - Met Thr Val Ala Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG CAC CCA AGT GAG AAT TGG CAA - Met Thr Val His Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG CAC CCA AGT GAG AAT TGG CAA - Met Thr Val His Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GAC CCA AGT GAG AAT TGG CAA - Met Thr Val Asp Pro Ser Glu -  
CG CTG CTC TTG CTG TCA CTA TTT AAG GTC ATG ACT GTG GCA CCA AGT GAG AAT TGG CAA - Met Thr Val Ala Pro Ser Glu -

**Supplementary Table S5.** L-Glutamate accumulation in the culture supernatant of constructed *mscCG* deletion mutants.

<i>C. glutamicum</i> variant	Sampling time [h] and L-glutamate concentration [mM]					
	14 h	16 h	18 h	20 h	22 h	24 h
wild type	0	0	0	0	0	0
M420stopΔ Clone 1	27.1		24	24.6	2.7	
M420stopΔ Clone 2	35.2	45.3	32.2	15.1	5.7	0.3
P424stopΔ Clone 1	42.4	89.1	82.6	61.4	53.6	59.5
P424stopΔ Clone 2	57.5	135.1	103.8	67.8	74.3	85.2
P424stopΔ Clone 3	62.1	97	91.1	47.7	57.4	33

## References

- (1) Schäfer, A.; Tauch, A.; Jäger, W.; Kalinowski, J.; Thierbach, G.; Pühler, A. Small Mobilizable Multi-Purpose Cloning Vectors Derived from the Escherichia Coli Plasmids PK18 and PK19: Selection of Defined Deletions in the Chromosome of *Corynebacterium Glutamicum*. *Gene* **1994**, *145* (1), 69–73. [https://doi.org/10.1016/0378-1119\(94\)90324-7](https://doi.org/10.1016/0378-1119(94)90324-7).
- (2) Hoffelder, M.; Raasch, K.; van Ooyen, J.; Eggeling, L. The E2 Domain of OdhA of *Corynebacterium Glutamicum* Has Succinyltransferase Activity Dependent on Lipoyl Residues of the Acetyltransferase AceF. *J. Bacteriol.* **2010**, *192* (19), 5203–5211. <https://doi.org/10.1128/JB.00597-10>.
- (3) Jiang, Y.; Qian, F.; Yang, J.; Liu, Y.; Dong, F.; Xu, C.; Sun, B.; Chen, B.; Xu, X.; Li, Y.; et al. CRISPR-Cpf1 Assisted Genome Editing of *Corynebacterium Glutamicum*. *Nat. Commun.* **2017**, *8*, 15179. <https://doi.org/10.1038/ncomms15179>.