

**Table S1.** Plasmids used in this study.

Plasmids	Relevant characteristics	Source or reference
pET24a	Km <sup>R</sup> , T7 promoter, T7 terminator	Lab stock
pET24a-tac	T7 promoter was replaced with tac promoter, T7 tag and 6×His tag was deleted	This study
pET24a- <i>panD</i>	the T7 tag and 6×His tag of pET24a-tac was replaced with the <i>panD</i> gene from <i>Corynebacterium glutamicum</i>	This study
pET24a- <i>AspDH</i>	the T7 tag and 6×His tag of pET24a-tac was replaced with the <i>AspDH</i> gene from <i>Klebsiella pneumoniae</i>	This study
pUC19	Amp <sup>r</sup>	Lab stock
pUC19-FRT-Kan-FRT	the cassette of FRT-Kan-FRT was amplified from pKD13 plasmid and cloned onto the plasmid of pUC19	This study
pUC19-FRT-Kan-FRT- <i>mhpB</i>	<i>mhpB</i> gene was amplified and cloned onto the plasmid of pUC19-FRT-Kan-FRT	This study
pUC19-FRT-Kan-FRT- <i>mhpB</i> <sup>*</sup>	<i>mhpB</i> gene containing all genic mutations listed in the genic mutation library	This study
pUC19-FRT-Kan-FRT- <i>mhpD</i>	<i>mhpD</i> gene was amplified and cloned onto the plasmid of pUC19-FRT-Kan-FRT	This study
pUC19-FRT-kan-FRT- <i>mhpD</i> <sup>*</sup>	<i>mhpD</i> gene containing all genic mutations listed in the genic mutation library	This study
pUC19-FRT-Kan-FRT- <i>rcsA</i>	<i>rcsA</i> gene was amplified and cloned onto the plasmid of pUC19-FRT-Kan-FRT	This study
pUC19-FRT-kan-FRT- <i>rcsA</i> <sup>*</sup>	<i>rcsA</i> gene containing all genic mutations listed in the genic mutation library	This study
pET24a- <i>panD</i> - <i>AspDH</i>	pET24a-tac containing the <i>panD</i> gene from <i>Corynebacterium glutamicum</i> and the <i>AspDH</i> gene from <i>Klebsiella pneumoniae</i>	This study
pET24a- <i>gfp</i>	pET24a containing <i>gfp</i> gene	This study
pET24a-tac- <i>gfp</i>	pET24a-tac containing <i>gfp</i> gene	This study
pKD46	Amp <sup>r</sup> , $\gamma$ βexo (red recombinase), Temperature-conditional replicon	CGSC <sup>a</sup> [40]
pKD13	Amp <sup>r</sup> , Kan <sup>r</sup> , FRT-Kan-FRT cassette	CGSC <sup>a</sup> [40]
pCP20	Amp <sup>r</sup> , FLP, Temperature-conditional replicon	CGSC <sup>a</sup> [40]

<sup>a</sup> Single-nucleotide variant. <sup>b</sup> Deletion mutation. <sup>c</sup> Insertion mutation. <sup>d</sup> Amino acid mutations.

**Table S2.** Primers used in this study.

Primers	Sequence (5'-3') restriction <i>italic/underlined</i>	Restriction sites
<i>ppc</i> -pKD13F	GAAGGATACAGGGCTATCAAACGATAAG ATGGGGTGTCTGGGGTAATATGGTGTAGG CTGGAGCTGCTTC	
<i>ppc</i> -pKD13R	AAAGCACGAGGGTTTGCAGAAGAGGAA GATTAGCCGGTATTACGCATACCATTCCG GGGATCCGTCGACC	

<i>YppcF</i>	GTAGAGCAGTGCCCAACCCAGG
<i>YppcR</i>	TTTGCTGAAGCGATTTTCGCAG
<i>recE</i> -pKD13F	GCAGATCGGCTTTTTCGATTGGTGGTTGC TTAGTCATTTGCATATTCCTTGTGTAGGCT GGAGCTGCTTC
<i>recE</i> -pKD13R	ATTCAGTGAACAAAACGAATTTTAATCTG AGTTGAGGTTAAAAACAATGATTCCGG GGATCCGTCGACC
<i>YrecEF</i>	CCGAGCTGTGAACACTGTACGAT
<i>YrecER</i>	CACTGTGGTTGATATCGATGGTCC
<i>mhpF</i> -pKD13F	TCAGTTGCTGCGACATTTTCAAGCGCAGC CCAAAAGGAAGTCTGTTCATGGTGTAGG CTGGAGCTGCTTC
<i>mhpF</i> -pKD13R	GACGTCCGAGATATAAAGTTTTTTACCGT TCATGCCGCTTCTCCTGCCTTATCCGGGG ATCCGTCGACC
<i>Y mhpF</i>	CAAAATGGCCAGTCTGGGTGAAC
<i>Y mhpFR</i>	CACTTCAATCGAATCCACGCG
<i>ykgF</i> -pKD13F	AGTGATGCATATTGCTGAAGTGTGATGA GCCGCTGAGGATATAAAGATGGTGTAGG CTGGAGCTGCTTC
<i>ykgF</i> -pKD13R	GAGCAACGTTATTCAAAAATTCGCCTCGA TTATCCATTCCTTTCTCCIGATTCCGGGG ATCCGTCGACC
<i>YykgFF</i>	TCAAAATGGCCGAAATATCCG
<i>YykgFR</i>	AACATAACATCGCTGGCAAACCTG
<i>mhpB</i> -pUC19F	ATGCACGCTTATCTTCACTGTCTTTCCAC TCGCCGCTGGTGGGGTATGTGTGTAGGCT GGAGCTGCTTC
<i>mhpB</i> -pUC19R	TCAGTTCTCTGTTCTGGCGCTTAACGAGC CAAATCCGGCAATCCACTCCG
<i>YmhpBF</i>	ATTCATACCGCACAGGATAACCAC
<i>YmhpBR</i>	AACCATGCAGCAGGACAACG
<i>mhpD</i> -pUC19F	ATGACGAAGCATACTTTGAGCAACTGG CGGCGGATTTACGCCGCGCCGCGTGTAG GCTGGAGCTGCTTC
<i>mhpD</i> -pUC19R	TCATGACAGACTTCCTTTGGGGCTGCGC TTGAAAATGTTCGAGCAACTG
<i>YmhpDF</i>	GCCTGGAAGCTAATCCGAAACA
<i>YmhpDR</i>	TCAGGCATGTTTCATCAGTCCG
<i>aspA</i> -pKD13F	GCCTTTTTTATTTGTACTACCCTGTACGAT TACTGTTTCGCTTTCATCAGTGTGTAGGCTG GAGCTGCTTC
<i>aspA</i> -pKD13R	CTGTGTGTTTAAAGCAAATCATTGGCAGC TTGAAAAAGAAGGTTACATGATTCCGG GGATCCGTCGACC
<i>YaspAF</i>	CCAATAGCGGCAAGAACCAG
<i>YaspAR</i>	CGAATCGGTTTTAGCTTATATTGTG
<i>rcsA</i> -pUC19F	ATGTCAACGATTATTATGGATTTATGTAG TTACACCCGACTAGGTTTAAACGTGTAGGC TGGAGCTGCTTC

<i>rcsA</i> -pUC19R	TTAGCGCATGTTGACAAAAATACCATTAG TCACATTATCCGTCAGTCGGA	
<i>YrcsAF</i>	AAGCTCACTCACATATCGCAACAT	
<i>YrcsAR</i>	GGTACTGGCAGTTGAGGAGTTTAGTG	
HindIII-FRT-Kan-FRTF	TTT <u>AAGCTT</u> <sup>a</sup> GTGTAGGCTGGAGCTGCTTC	HindIII <sup>a</sup>
HindIII-FRT-Kan-FRTR	TTT <u>AAGCTT</u> <sup>a</sup> ATTCCGGGGATCCGTCGACC	HindIII <sup>a</sup>
BamHI- <i>mhpBF</i>	TTT <u>GGATCC</u> <sup>a</sup> ATGCACGCTTATCTTCACTGT CTTTC	BamHI <sup>a</sup>
EcoRI- <i>mhpBR</i>	TTT <u>GAATTC</u> <sup>a</sup> TCAGTTCTCTGTTCTGGCGCT TAA	EcoRI <sup>a</sup>
<i>mhpB</i> *F	ATTGCCAGCGCCCGCGCGCTATTGCGG	
<i>mhpB</i> *R	GAGAATGCCGCAATACGCGCGCGGGCGC T	
BamHI- <i>mhpDF</i>	TTT <u>GGATCC</u> <sup>a</sup> ATGACGAAGCATACTCTTGA GCAACT	BamHI <sup>a</sup>
EcoRI- <i>mhpDR</i>	TTT <u>GAATTC</u> <sup>a</sup> TCATGACAGACTTCCTTTTGG GG	EcoRI <sup>a</sup>
<i>mhpD</i> *F	TGTGTTATGGCGATAACGAAACCATTCTT TTTT	
<i>mhpD</i> *R	GTTGCAGAACACGGGAAAAAAGGAATGGT TTCGTTATC	
BamHI- <i>rscAF</i>	TTT <u>GGATCC</u> <sup>a</sup> ATGTCAACGATTATTATGGA TTTATGTAGTTAC	BamHI <sup>a</sup>
EcoRI- <i>rscAR</i>	TTT <u>GAATTC</u> <sup>a</sup> TTAGCGCATGTTGACAAAAA TACC	EcoRI <sup>a</sup>
<i>rscA</i> *F	TCTAACAGTCAGCGTATCAAGCACATCAT TAATCAA	
<i>rscA</i> *R	TATTGGGATGTTGATTAATGATGTGCTTG ATACGC	
pET24a- <i>tacF</i>	TCCGCTCACAATCCCATTATACGAGCCG ATGATTAATTGTCAAATTCGCGGGATCG AGATCTC	
pET24a- <i>tacR</i>	TCGGCTCGTATAATGGGAATTGTGAGCGG ATAACAATTCC	
<i>panD</i> - pET24aF	AATGGTGCGCAGCATATGTATATCTCCTT CTTAAAGTTAAACAAAATTATTTCTAGA	
<i>panD</i> - pET24aR	AGCCGCAGCATCTAACCCGAAAGGAAGC TGAGTTGG	
pET24a- <i>panDF</i>	GAAGGAGATATACATATGCTGCGCACCA TTCTGG	
pET24a- <i>panDR</i>	CAGCTTCCTTTTCGGGTTAGATGCTGCGGC TGGTCAAG	
<i>AspDH</i> - pET24aF	CACTTTCTTCATCATATGTATATCTCCTTC TTAAAGTTAAACAAAATTATTTCTAGA	
<i>AspDH</i> - pET24aR	CGTGAAGTGGCCTAACCCGAAAGGAAGC TGAGTTGG	
pET24a- <i>AspDHF</i>	GAAGGAGATATACATATGATGAAGAAAG TGATGCTGATCG	
pET24a- <i>AspDHR</i>	CAGCTTCCTTTTCGGGTTAGGCCAGTTCAC GGCAC	

<i>panD</i> - pET24a- <i>AspDHF</i>	GCGGGATCGAGATCTGAAGATCGGGCTC GCCACTT	
<i>panD</i> - pET24a- <i>AspDHR</i>	TTGAGGGGTTTTTTTTCGGTGATGTCGGCG ATATAGGC	
pET24a- <i>AspDH</i> - <i>panDF</i>	GCGAGCCCGATCTTCAGATCTCGATCCCG CGAAAT	
pET24a- <i>AspDH</i> - <i>panDR</i>	TCGCCGACATCACCGCAAAAACCCCTC AAGACCCG	
<i>gfp</i> -BamHIF	TTT <u>GGATCC</u> <sup>a</sup> ATGAGTAAAGGAGAAGAAC TTTTCACTGG	BamHI <sup>a</sup>
<i>gfp</i> -EcoRIR	TTT <u>GAATTC</u> <sup>a</sup> TTATTTGTATAGTTCATCCAT GCCATGT	EcoRI <sup>a</sup>
Ypet24aF	ATCATGCCATACCGCGAAAGG	
Ypet24aR	GCTTAATGCGCCGCTACAGG	

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<sup>a</sup> Restriction sites with corresponding restriction enzymes. \* Primer of site-directed mutagenesis.