# Reconstruction of the diaminopimelic acid pathway to promote L-lysine production in *Corynebacterium glutamicum*

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**The build processes of recombinant plasmids**

The plasmid pEC-XK99E-*ddh* construction

The nucleotide sequences of the gene *ddh* from *C. glutamicum* XQ-5 with two restriction sites (i.e., *Eco*RI and *Xba*I) were amplified by PCR, and then ligated with plasmid pEC-XK99E. The resulted plasmid was designated as pEC-XK99E-*ddh*.

The plasmid pK18*mobsacB*-Δ*amtR* construction

The homology arm sequences of the gene *amtR* from *C. glutamicum* XQ-5 with two restriction sites (i.e., *Eco*RI and *Xba*I) were amplified by fusion PCR, and then ligated with plasmid pK18*mobsacB*. The resulted plasmid was designated as pK18*mobsacB*-Δ*amtR*.

The plasmid pK18*mobsacB*-Δ*dapD* construction

The homology arm sequences of the gene *dapD* from *C. glutamicum* XQ-5 with two restriction sites (i.e., *Sma*I and *Hin*dIII) were amplified by fusion PCR, and then ligated with plasmid pK18*mobsacB*. The resulted plasmid was designated as pK18*mobsacB*-Δ*dapD*.

The plasmid pK18*mobsacB*-Δ*ddh* construction

The homology arm sequences of the gene *ddh* from *C. glutamicum* XQ-5 with two restriction sites (i.e., *Eco*RI and *Xba*I) were amplified by fusion PCR, and then ligated with plasmid pK18*mobsacB*. The resulted plasmid was designated as pK18*mobsacB*-Δ*ddh*.

The plasmid pK18*mobsacB*-T1, T2, T3, T4, T5, T6 construction

The T1 terminator and the left homologous arm of gene *dapD* were designed on the same primer, and then the gene *dapD* homologous arm sequences were amplified by fusion PCR, and then ligated

with plasmid pK18*mobsacB*. The resulted plasmid was designated as pK18*mobsacB*-T1. Construction of plasmid pK18mobsacB- T2, T3, T4, T5, T6 were similar to that of plasmid pK18*mobsacB*-T1.

**The procedures of recombinant strain constructions**

Construction of XQ-5-2 (Δ*amtR*), XQ-5-4 (Δ*dapD*), XQ-5-5 (Δ*ddh*) and XQ-5-6 (Δ*amtR*Δ*dapD*)

The plasmid pK18*mobsacB*-Δ*amtR*, pK18*mobsacB*-Δ*ddh and* pK18*mobsacB*-Δ*dapD* were electroporated into *C. glutamicum* XQ-5 , and then the positive transformants were screened with 25μg /mL concentration of kanamycin in LBH medium. The final positive transformants were obtained by eliminating the plasmids according to the sucrose lethal principle. The deletion of genes *amtR*, *ddh* and *dapD* were analyzed by PCR, and strain XQ-5-2, XQ-5-4 and XQ-5-5 were obtained. The strain XQ-5-6 was based on strain XQ-5-2 by deleting the gene dapD through the same steps.

Construction of XQ-5-1 (XQ-5/pEC-*ddh*), XQ-5-3 (Δ*amtR*/pEC-*ddh*), XQ-5-7 (Δ*dapD*/pEC-*ddh*) and XQ-5-8 (Δ*amtR*Δ*dapD* /pEC-*ddh*)

The plasmid pEC-XK99E-*ddh* was transformed into XQ-5, XQ-5-2, XQ-5-4 and XQ-5-6, respectively, and then resulting in XQ-5-1, XQ-5-3, XQ-5-7 and XQ-5-8, respectively.

Construction of XQ-5-W1, XQ-5-W2, XQ-5-W3, XQ-5-W4, XQ-5-W5 and XQ-5-W6.

The plasmid pK18*mobsacB*-T1, pK18*mobsacB*-T2, pK18*mobsacB*-T3, pK18*mobsacB*-T4, pK18*mobsacB*-T5 and pK18*mobsacB*-T6 were electroporated into strain XQ-5-3 (Δ*amtR*/pEC-*ddh*), and then the positive transformants were screened with 25μg /mL concentration of kanamycin in LBH medium. The final positive transformants were obtained by eliminating the plasmids according to the sucrose lethal principle. The strain XQ-5-W1, XQ-5-W2, XQ-5-W3, XQ-5-W4, XQ-5-W5 and XQ-5-W6 were obtained by PCR analysis.

**Table S1** The Sequence of Terminators

| name | terminator sequence (5'-3') | strength |
|---|---|---|
| T1 | CCAATTATTGAAGCGGCTAACGCCGCTTTTTTTGTTTCTGGT | 67.43 |
| T2 | CCAATTATTGAACACCCTAACGGGTGTTTTTTTGTTTCTGGT | 128.86 |
| T3 | GACGAACAATAAGGCCTCCCTAACGGGGGGCCTTTTTTATT | 177.94 |
| T4 | CTCGGTACCAAATTCCAGAAAAGAGACGCTGAAAAGCGTC | 239.91 |
| T5 | CTCGGTACCAAAAAAAAAAAAAAAGACACTGAAAAGCGT | 309.20 |
| T6 | GGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCT | 382.13 |

**Table S2** The oligonucleotides used in this study

| **primer** | **sequence (5'-3')** | restriction sites |
|---|---|---|
| *amtR*-L-F | CCGGAATTCGAATCACTCGTGCTTCACCCC | *Eco*RI |
| *amtR*-L-R | TTGGCCTATTGATCTCAAGAGATTGTAGCCAGTA | |
| *amtR*-R-F | AGATCAATAGGCCAACTCGCCACCGAAATCGT | |
| *amtR*-R-R | TGCTCTAGAGTGCAGCCTGGTGGATCCATT | *Xba*I |
| *dapD*-L-F | TCCCCCGGGGAACCGTGGATTCCTTCGCT | *Sma*I |
| *dapD* -L-R | GAATAATTTCTATCGGCCTATCAGTTCAAGT | |
| *dapD* -R-F | TAGAAATTATTCCCTAGCCTGGATCCGATC | |
| *dapD* -R-R | CCCAAGCTTCTACCTCGCCTCCATCATGAT | *Hind*III |
| *ddh*-L-F | CCGGAATTCTCACGGATGCTGTTGGGCA | *Eco*RI |
| *ddh*-L-R | TTGTAATCCTCCAAAATTGTGGTGGCACTGTC | |
| *ddh*-R-F | TTTGGAGGATTACAATCGAGGGGCAAGGAAACA | |
| *ddh*-R-R | TGCTCTAGACGTCACAAACCCATCTTTAG | *Xba*I |
| pEC-*ddh*-F | CCGGAATTCCGTTCGCTTTCGGTCCTGAT | *Eco*RI |
| pEC-*ddh*-R | CTAGTCTAGAGTCACGCCTGTCTGGTTTCCT | *Xba*I |
| *dapD*$^{W1}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *Eco*RI |
| *dapD*$^{W1}$-L-R | ACCAGAAACAAAAAAAGCGGCGTTAGCCGCTTC<br>AATAATTGGCCTATCAGTTCAAGTCGGAAGG | |
| *dapD*$^{W1}$-R-F | AGCGGCTAACGCCGCTTTTTTTGTTTCTGGTCCG<br>ATAGAAATTATTCTGGACGTC | |
| *dapD*$^{W1}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |
| *dapD*$^{W2}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *Eco*RI |
| *dapD*$^{W2}$-L-R | CCAGAAACAAAAAAACACCCGTTAGGGTGTTCA<br>ATAATTGGCCTATCAGTTCAAGTCGGAAGG | |
| *dapD*$^{W2}$-R-F | ACACCCTAACGGGTGTTTTTTTGTTTCTGGTCCGA<br>TAGAAATTATTCTGGACGTC | |
| *dapD*$^{W2}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |
| *dapD*$^{W3}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *Eco*RI |
| *dapD*$^{W3}$-L-R | AATAAAAAAGGCCCCCCGTTAGGGAGGCCTTATTG<br>TTCGTCCCTATCAGTTCAAGTCGGAAGG | |
| *dapD*$^{W3}$-R-F | AGGCCTCCCTAACGGGGGGCCTTTTTTATTCCGATA<br>GAAATTATTCTGGACGTC | |
| *dapD*$^{W3}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |
| *dapD*$^{W4}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *Eco*RI |
| *dapD*$^{W4}$-L-R | GACGCTTTTCAGCGTCTCTTTTCTGGAATTTGGTAC<br>CGAGCCTATCAGTTCAAGTCGGAAGG | |
| *dapD*$^{W4}$-R-F | AATTCCAGAAAAGAGACGCTGAAAAGCGTCCCGA<br>TAGAAATTATTCTGGACGTC | |
| *dapD*$^{W4}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |
| *dapD*$^{W5}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *Eco*RI |
| *dapD*$^{W5}$-L-R | ACGCTTTTCAGTGTCTTTTTTTTTTTTTTGGTACCG<br>AGCCTATCAGTTCAAGTCGGAAGG | |

**Refer to Table S2 (continued)**

| **primer** | **sequence (5'-3')** | restriction sites |
|---|---|---|
| *dapD*$^{W5}$-R-F | AAAAAAAAAAAAAAAAGACACTGAAAAGCGTCCGA | |
| | TAGAAATTATTCTGGACGTC | |
| *dapD*$^{W5}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |
| *dapD*$^{W6}$-L-F | CCGGAATTCCGCTTTCCTCATCATTGGT | *EcoR*I |
| *dapD*$^{W6}$-L-R | GCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTT | |
| | CCCCTATCAGTTCAAGTCGGAAG | |
| *dapD*$^{W6}$-R-F | GAAAAAAGCCCGCACCTGACAGTGCGGGCTCCGAT | |
| | AGAAATTATTCTGGACGTC | |
| *dapD*$^{W6}$-R-R | CCCAAGCCTGTCCGTTGGTGAAAGCGAT | *Hind*III |

**Table S3** Primers used for RT-PCR in this work

| primers | sequence (5'-3') |
|---|---|
| 16SRNA-F: | ACCTGGAGAAGAAGCACCG |
| 16SRNA-R | TCAAGTTATGCCCGTATCG |
| *ddh*-F | CGTGCTGTTCCTGTGCATG |
| *ddh*-R | AGAACATTCCTGGATCCCAGC |
| *dapD*-F | GCCTCCTCAACAATGTCGTG |
| *dapD*-R | CACGAAGCCCTCATGCATCA |