**Designing of an efficient whole-cell biocatalyst** **system for converting L-lysine into cis-3-hydroxypipecolic acid**

Shewei Hu, Yangyang Li, Alei Zhang, Hui Li, Kequan Chen\*, Pingkai Ouyang

*State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, 211800, P.R. China*

\*Corresponding author: Tel.: +86-138-1418-0652

E-mail address: kqchen@njtech.edu.cn

Table S1 *E. coli* strains and plasmids used in this study

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| strains or plasmids | Relevant characteristics | Source |
| Strains |  |  |
| *E. coli* DH5α | Cloning host | Novagen |
| *E. coli* BL21(DE3) | Protein expression host | Novagen |
| *E. coli* DB3.1 | Cloning host for pEcgRNA | Shanghai Institutes for Biological Sciences |
| Strain1 | *E. coli* BL21(DE3) harboring plasmid 1 | This lab |
| Strain2Strain3Strain4Strain5Strain6Strain7Strain8Strain9Strain10Strain11Strain12Strain13Strain14Strain15 | *E. coli* BL21(DE3) harboring plasmid 2*E. coli* BL21(DE3) harboring plasmid 3*E. coli* BL21(DE3) harboring plasmid 4*E. coli* BL21(DE3) harboring plasmid 5*E. coli* BL21(DE3) harboring plasmid 6*E. coli* BL21(DE3) harboring plasmid 7*E. coli* BL21(DE3) harboring plasmid 8*E. coli* BL21(DE3) harboring plasmid 9*E. coli* BL21(DE3) harboring plasmid 10*E. coli* BL21(DE3) harboring plasmid 11*E. coli* BL21(DE3) Δ*sucA*harboring plasmid 2*E. coli* BL21(DE3) Δ*aceA*harboring plasmid 2*E. coli* BL21(DE3) Δ*sucA*Δ*aceA* harboring plasmid 2*E. coli* BL21(DE3) Δ*suc*AΔ*aceA* harboring plasmid 2 and plasmid 12 | This studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis study |
| Plasmids pCDFDuet-1 | double T7 promoters, CDF 13 ori, SmR | Novagen |
| pETDuet-1 | double T7 promoters, pBR322 ori, AmpR | Novagen |
| Plasmid1 Plasmid2Plasmid3Plasmid4Plasmid5Plasmid6Plasmid7Plasmid8Plasmid9Plasmid10Plasmid11Plasmid12Plasmid13Plasmid14Plasmid15Plasmid16 Plasmid17Plasmid18 | pETDuet-1 with RBS T7 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 030 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 31 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 032 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 064 linked with GetF and RBS T7 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS 029 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS 030 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS 031 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS 32 with SpLCDpETDuet-1 with RBS 029 linked with GetF and RBS 064 with SpLCDpCDFDuet-1with glta and icdpEcCas pEcgRNApEcgRNA -*aceA*-N20A pEcgRNA- *aceA*-N20BpEcgRNA -*sucA*-N20ApEcgRNA -*sucA*-N20B | This labThis studyThis studyThis studyThis study This studyThis studyThis studyThis studyThis studyThis studyThis studyShanghai Institutes for Biological SciencesShanghai Institutes for Biological SciencesThis studyThis studyThis studyThis study |

Table S2 Primers for construction of various RBS site used in this study

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| Plasmid2F ACTTTAAGTTCACACAGGAAACCATATACCATGGGCAGCAGCCATCACCATCPlasmid2RGGTATATGGTTTCCTGTGTGAACTTAAAGTTAAACAAAATTATTTCTAGAGGG |

Plasmid3F

ACTTTAAGATTAAAGAGGAGAAAATATACCATGGGCAGCAGCCATCACCATC

Plasmid3R

GGTATATTTTCTCCTCTTTAATCTTAAAGTTAAACAAAATTATTTCTAGAGGG

Plasmid4F

ACTTTAAG TCACACAGGAAACC ATATACCATGGGCAGCAGCCATCACCATC

Plasmid4R

GGTATAT TTTCTCCTCTTTAATCTTAAAGTTAAACAAAATTATTTCTAGAGGG

Plasmid5F

ACTTTAAG TCACACAGGAAAG ATATACCATGGGCAGCAGCCATCACCATC

Plasmid5R

GGTATAT CTTTCCTGTGTGACTTAAAGTTAAACAAAATTATTTCTAGAGGG

Plasmid6F

ACTTTAAG AAAGAGGGGAAAATATACCATGGGCAGCAGCCATCACCATC

Plasmid6R

GGTATAT TTTCCCCTCTTTCTTAAAGTTAAACAAAATTATTTCTAGAGGG

Plasmid7F

AGTATAAGTTCACACAGGAAACCATATACATATGGCAGAT

Plasmid7R

CATATGTATATGGTTTCCTGTGTGAACTTATACTTAACTAATATACTAA

Plasmid8F

AGTATAAGATTAAAGAGGAGAAAATATACATATGGCAGAT

Plasmid8R

CATATGTATATTTTCTCCTCTTTAATCTTATACTTAACTAATATACTAA

Plasmid9F

AGTATAAG TCACACAGGAAACC ATATACATATGGCAGAT

Plasmid9R

CATATGTATATTTTCTCCTCTTTAATCTTATACTTAACTAATATACTAA

Plasmid10F

AGTATAAG TCACACAGGAAAG ATATACATATGGCAGAT

Plasmid10R CATATGTATATCTTTCCTGTGTGACTTATACTTAACTAATATACTAA

Plasmid11F AGTATAAGAAAGAGGGGAAAATATACATATGGCAGAT

Plasmid11R CATATGTATATTTTCCCCTCTTTCTTATACTTAACTAATATACTAA

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Table S3 Primers for gene editing used in this study. DNA fragment (5′–3′)

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| *aceA* F1 CCTGCCATAAACGCGGTGC |

*aceA* R1 TTGTTGTTGCGTGCAGATGCTCCATAGTTATGTG

*aceA* F2 GCATCTGCACGCAACAACAACCGTTGCTG

*aceA*R2 CGCCACCTGTAAATGACTTTCC

*aceA*F3 ATCGCGTCCTGCCAGACAGAC

*aceA*R3 GGTGTGATCAGTTTGCCTACCAGC

*aceA*-N20A-Fa TAGTCGTCTGGCAGCTGACGTGAC

*aceA*-N20A-Ra AAACGTCACGTCAGCTGCCAGACG

*aceA*-N20B-Fb TAGTGCTTCCGCATCGGCAACGAT

*aceA*-N20B-Rb AAACATCGTTGCCGATGCGGAAGC

*sucA*F1 AGAAGATTGTGATTCGCCCGC

*sucA*R1 TTGTGTATCCTTTATCGTGATCCCTTAAGCATCTTTTT

*sucA*F2 CTTAAGGGATCACGATAAAGGATACACAATGAGTAGCGTAGA

*sucA*R2 GCTTCAACCACCGCTTTCACG

*sucA*F3 GATGAAGGTCGCGACATGATGCT

*sucA*R3 TGCTGACGTCGAAATAGTTGTGG

*sucA*-N20A-Fa TAGTTTACCGATATCAGTACAGTA

*sucA*-N20A-Ra AAACTACTGTACTGATATCGGTAA

*sucA*-N20B-Fb TAGT GCGATTGCGTAGTGGCAGAG

*sucA*-N20B-Rb AAAC CTCTGCCACTACGCAATCGC

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Table S4 Primers for construction plasmid pCDFduet-*glta*-*icd* used in this study. DNA fragment (5′–3′)

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| glta(*EcoR I*) CGGAATTCatggctgatacaaaagcaaaactcglta(*Hind III*) cccaagcttttaacgcttgatatcgcttttaaagtcicd(*Bgl II*) gaagatctatggaaagtaaagtagttgttccg |

icd(*Xho I*) ccgctcgagttacatgttttcgatgatcgcgt

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Table S5 Donor DNA sequence Homologous DNA fragment (5′–3′)

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| *aceA* |

CCTGCCATAAACGCGGTGCTTTTGCGATGGGCGGCATGGCGGCGTTTATTCCGAGCAAAGATGAAGAGCACAATAACCAGGTGCTCAACAAAGTAAAAGCGGATAAATCGCTGGAAGCCAATAACGGTCACGATGGCACATGGATCGCTCACCCAGGCCTTGCGGACACGGCAATGGCGGTATTCAACGACATTCTCGGCTCCCGTAAAAATCAGCTTGAAGTGATGCGCGAACAAGACGCGCCGATTACTGCCGATCAGCTGCTGGCACCTTGTGATGGTGAACGCACCGAAGAAGGTATGCGCGCCAACATTCGCGTGGCTGTGCAGTACATCGAAGCGTGGATCTCTGGCAACGGCTGTGTGCCGATTTATGGCCTGATGGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGTTCCGCCAGATGCTGGGCGAAGAGATGAAAGTCATTGCCAGCGAACTGGGCGAAGAACGTTTCTCCCAGGGGCGTTTTGACGATGCCGCACGCTTGATGGAACAGATCACCACTTCCGATGAGTTAATTGATTTCCTGACCCTGCCAGGCTACCGCCTGTTAGCGTAAACCACCACATAACTATGGAGCATCTGCACGCAACAACAACCGTTGCTGACTGTAGGCCGGATAAGGCGTTCACGCCGCATCCGGCAATCGGTGCACGATGCCTGATGCGACGCTTGCGCGTCTTATCATGCCTACAGCCGTTGCCGAACGTAGGCTGGATAAGGCGTTTACGCCGCATCCGGCAATTCTCTGCTCCTGATGAGGGCGCTAAATGCCGCGTGGCCTGGAATTATTGATTGCTCAAACCATTTTGCAAGGCTTCGATGCTCAGTATGGTCGATTCCTCGAAGTGACCTCCGGTGCGCAGCAGCGTTTCGAACAGGCCGACTGGCATGCTGTCCAGCAGGCGATGAAAAACCGTATCCATCTTTACGATCATCACGTTGGTCTGGTCGTGGAGCAACTGCGCTGCATTACTAACGGCCAAAGTACGGACGCGGCATTTTTACTACGTGTTAAAGAGCATTACACCCGGCTGTTGCCGGATTACCCGCGCTTCGAGATTGCGGAGAGCTTTTTTAACTCCGTGTACTGTCGGTTATTTGACCACCGCTCGCTTACTCCCGAGCGGCTTTTTATCTTTAGCTCTCAGCCAGAGCGCCGCTTTCGTACCATTCCCCGCCCGCTGGCGAAAGACTTTCACCCCGATCACGGCTGGGAATCTCTACTGATGCGCGTTATCAGCGACCTACCGCTGCGCCTGCGCTGGCAGAATAAAAGCCGTGACATCCATTACATTATTCGCCATCTGACGGAAACGCTGGGGACAGACAACCTCGCGGAAAGTCATTTACAGGTGGCG

*sucA*

 AGAAGATTGTGATTCGCCCGCTGCCAGGTTTACCGGTGATCCGCGATTTGGTGGTAGACATGGGACAATTCTATGCGCAATATGAGAAAATTAAGCCTTACCTGTTGAATAATGGACAAAATCCGCCAGCTCGCGAGCATTTACAGATGCCAGAGCAGCGCGAAAAACTCGACGGGCTGTATGAATGTATTCTCTGCGCATGTTGTTCAACCTCTTGTCCGTCTTTCTGGTGGAATCCCGATAAGTTTATCGGCCCGGCAGGCTTGTTAGCGGCATATCGTTTCCTGATTGATAGCCGTGATACCGAGACTGACAGCCGCCTCGACGGTTTGAGTGATGCATTCAGCGTATTCCGCTGTCACAGCATCATGAACTGCGTCAGTGTATGTCCGAAGGGGCTGAACCCGACGCGCGCCATCGGCCATATCAAGTCGATGTTGTTGCAACGTAATGCGTAAACCGTAGGCCTGATAAGACGCGCAAGCGTCGCATCAGGCAACCAGTGCCGGATGCGGCGTGAACGCCTTATCCGGCCTACAAGTCATTACCCGTAGGCCTGATAAGCGCAGCGCATCAGGCGTAACAAAGAAATGCAGGAAATCTTTAAAAACTGCCCCTGACACTAAGACAGTTTTTAAAGGTTCCTTCGCGAGCCACTACGTAGACAAGAGCTCGCAAGTGAACCCCGGCACGCACATCACTGTGCGTGGTAGTATCCACGGCGAAGTAAGCATAAAAAAGATGCTTAAGGGATCACGATAAAGGATACACAATGAGTAGCGTAGATATTCTGGTCCCTGACCTGCCTGAATCCGTAGCCGATGCCACCGTCGCAACCTGGCATAAAAAACCCGGCGACGCAGTCGTACGTGATGAAGTGCTGGTAGAAATCGAAACTGACAAAGTGGTACTGGAAGTACCGGCATCAGCAGACGGCATTCTGGATGCGGTTCTGGAAGATGAAGGTACAACGGTAACGTCTCGTCAGATCCTTGGTCGCCTGCGTGAAGGCAACAGCGCCGGTAAAGAAACCAGCGCCAAATCTGAAGAGAAAGCGTCCACTCCGGCGCAACGCCAGCAGGCGTCTCTGGAAGAGCAAAACAACGATGCGTTAAGCCCGGCGATCCGTCGCCTGCTGGCTGAACACAATCTCGACGCCAGCGCCATTAAAGGCACCGGTGTGGGTGGTCGTCTGACTCGTGAAGATGTGGAAAAACATCTGGCGAAAGCCCCGGCGAAAGAGTCTGCTCCGGCAGCGGCTGCTCCGGCGGCGCAACCGGCTCTGGCTGCACGTAGTGAAAAACGTGTCCCGATGACTCGCCTGCGTAAGCGTGTGGCAGAGCGTCTGCTGGAAGCGAAAAACTCCACCGCCATGCTGACCACGTTCAACGAAGTCAACATGAAGCCGATTATGGATCTGCGTAAGCAGTACGGTGAAGCGTTTGAAAAACGCCACGGCATCCGTCTGGGCTTTATGTCCTTCTACGTGAAAGCGGTGGTTGAAGC

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| Table S6 icd and glta genes sequences and enzyme cleavage site |
| *glta* (*EcoR I*/*Hind III*) |

GAATTCatggctgatacaaaagcaaaactcaccctcaacggggatacagctgttgaactggatgtgctgaaaggcacgctgggtcaagatgttattgatatccgtactctcggttcaaaaggtgtgttcacctttgacccaggcttcacttcaaccgcatcctgcgaatctaaaattacttttattgatggtgatgaaggtattttgctgcaccgcggtttcccgatcgatcagctggcgaccgattctaactacctggaagtttgttacatcctgctgaatggtgaaaaaccgactcaggaacagtatgacgaatttaaaactacggtgacccgtcataccatgatccacgagcagattacccgtctgttccatgctttccgtcgcgactcgcatccaatggcagtcatgtgtggtattaccggcgcgctggcggcgttctatcacgactcgctggatgttaacaatcctcgtcaccgtgaaattgccgcgttccgcctgctgtcgaaaatgccgaccatggccgcgatgtgttacaagtattccattggtcagccatttgtttacccgcgcaacgatctctcctacgccggtaacttcctgaatatgatgttctccacgccgtgcgaaccgtatgaagttaatccgattctggaacgtgctatggaccgtattctgatcctgcacgctgaccatgaacagaacgcctctacctccaccgtgcgtaccgctggctcttcgggtgcgaacccgtttgcctgtatcgcagcaggtattgcttcactgtggggacctgcgcacggcggtgctaacgaagcggcgctgaaaatgctggaagaaatcagctccgttaaacacattccggaatttgttcgtcgtgcgaaagacaaaaatgattctttccgcctgatgggcttcggtcaccgcgtgtacaaaaattacgacccgcgcgccaccgtaatgcgtgaaacctgccatgaagtgctgaaagagctgggcacgaaggatgacctgctggaagtggctatggagctggaaaacatcgcgctgaacgacccgtactttatcgagaagaaactgtacccgaacgtcgatttctactctggtatcatcctgaaagcgatgggtattccgtcttccatgttcaccgtcattttcgcaatggcacgtaccgttggctggatcgcccactggagcgaaatgcacagtgacggtatgaagattgcccgtccgcgtcagctgtatacaggatatgaaaaacgcgactttaaaagcgatatcaagcgttaaaagctt

*icd* (*Bgl II* / *XhoI*)

agatctatggaaagtaaagtagttgttccggcacaaggcaagaagatcaccctgcaaaacggcaaactcaacgttcctgaaaatccgattatcccttacattgaaggtgatggaatcggtgtagatgtaaccccagccatgctgaaagtggtcgacgctgcagtcgagaaagcctataaaggcgagcgtaaaatctcctggatggaaatttacaccggtgaaaaatccacacaggtttatggtcaggacgtctggctgcctgctgaaactcttgatctgattcgtgaatatcgcgttgccattaaaggtccgctgaccactccggttggtggcggtattcgctctctgaacgttgccctgcgccaggaactggatctctacatctgcctgcgtccggtacgttactatcagggcactccaagcccggttaaacaccctgaactgaccgatatggttatcttccgtgaaaactcggaagacatttatgcgggtatcgaatggaaagcagactctgccgacgccgagaaagtgattaaattcctgcgtgaagagatgggggtgaagaaaattcgcttcccggaacattgtggtatcggtattaagccgtgttcggaagaaggcaccaaacgtctggttcgtgcagcgatcgaatacgcaattgctaacgatcgtgactctgtgactctggtgcacaaaggcaacatcatgaagttcaccgaaggagcgtttaaagactggggctaccagctggcgcgtgaagagtttggcggtgaactgatcgacggtggcccgtggctgaaagttaaaaacccgaacactggcaaagagatcgtcattaaagacgtgattgctgatgcattcctgcaacagatcctgctgcgtccggctgaatatgatgttatcgcctgtatgaacctgaacggtgactacatttctgacgccctggcagcgcaggttggcggtatcggtatcgcccctggtgcaaacatcggtgacgaatgcgccctgtttgaagccacccacggtactgcgccgaaatatgccggtcaggacaaagtaaatcctggctctattattctctccgctgagatgatgctgcgccacatgggttggaccgaagcggctgacttaattgttaaaggtatggaaggcgcaatcaacgcgaaaaccgtaacctatgacttcgagcgtctgatggatggcgctaaactgctgaaatgttcagagtttggtgacgcgatcatcgaaaacatgtaactcgag

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