Engineering *Escherichia coli* for glutarate production as C5 platform backbone

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Supplementary Table S1 Strains and plasmids used in this study

Name	Relevant genotype	Reference
Strains		
JM109	For plasmid construction	Prof. Zhou (1)
BL21(DE3)	For expressing genes	Prof. Zhou (1)
Bgl146	BL21(DE3) carrying pAD1, pAD4 and pAD6	This study
Bgl1	BL21(DE3) Δ*arcA*	This study
Bgl1146	Bgl1 carrying pAD1, pAD4 and pAD6	This study
Bgl4	BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB*	This study
Bgl4146	Bgl4 carrying pAD1, pAD4 and pAD6	This study
Plasmids		
pRSFDuet-1	RSF ori, lacI, T7 lac, Kan^R	Novagen(1)
pCDFDuet-1	CloDF13 ori, lacI, T7 lac, Strep^R	Novagen(1)
pTrc99a	pBR322 ori, trc, Amp^R	Novagen
pUC57-1	pUC57 harboring the optimized gene *Tfu-0875* from *Thermobifida fusca*, Amp^R	Genewiz
pUC57-2	pUC57 harboring the optimized gene *Tfu-2399* from *Thermobifida fusca*, Amp^R	Genewiz
pUC57-3	pUC57 harboring the optimized gene *Tfu-0067* from *Thermobifida fusca*, Amp^R	Genewiz
pUC57-4	pUC57 harboring the optimized gene *Tfu-1647* from *Thermobifida fusca*, Amp^R	Genewiz
pUC57-5	pUC57 harboring the optimized genes *Tfu-2576* and *Tfu-2577* from *Thermobifida fusca*, Amp^R	Genewiz
pRSF- Tfu_0875	pRSFDuet-1 harboring the optimized gene *Tfu-0875* from pUC57-1, Kan^R	This study

pAD1	pRSFDuet-1 harboring the optimized genes *Tfu-0875* and *Tfu-2399* from pUC57-1 and pUC57-2, Kan^R	This study
pAD4	pTrc99a harboring the optimized genes *Tfu-0067* and *Tfu-1647* from pUC57-3 and pUC57-4, Amp^R	This study
pAD6	pCDFDuet-1 harboring the optimized genes *Tfu-2576* and *Tfu-2577* from pUC57-5, Strep^R	This study
parcA	pRSFDuet-1 harboring the *arcA* gene *upstream* and downstream 500bp from *E. coli*, Kan^R	This study
pldhA	pRSFDuet-1 harboring the *ldhA* gene *upstream* and downstream 500bp from *E. coli*, Kan^R	This study
patoB	pRSFDuet-1 harboring the *atoB* gene *upstream* and downstream 500bp from *E. coli*, Kan^R	This study
ppflB	pRSFDuet-1 harboring the *pflB* gene *upstream* and downstream 500bp from *E. coli*, Kan^R	This study
pCas	paraB-gam-bet-exo, bla (kan^R), kanR-repA101 (ts),λ-Red, the sgRNA with a lacI^q-Ptrc promoter guiding the pMB1 replication of pTarget	Addgene(2)
pTarget	the sgRNA sequence, a targeting N20 sequence, the pMB1 replicon, Spe^R	Addgene(2)

Supplementary Table S2 Primers used in this study

Primer	Sequence (5' to 3')
pUC57-1 F(*Eco*R I)	CGGAATTCATGACCGATGTTTATATTCTGGA
pUC57-1 R(*Hin*d III)	CCCAAGCTTTTAACGATGCAGAACGACGG
pUC57-2 F(*Bgl* II)	GAAGATCTATGGTTGAGGAAATTAATAAAG
pUC57-2 R(*Kpn* I)	GGGGTACCTTAACTCCGCAGAGTGTCCT
pUC57-3 F(*Nco* I)	CATGCCATGGATGGGTGAATTTATCCGCTTTG
pUC57-3 R(*Hin*d III)	CCCAAGCTTTTATTCTTTTTTATTTTTGCGCGCC
pUC57-4 F(*Nde* I)	GGGAATTCCATATGATGTCTGATTTTGATCTGTACCGG
pUC57-4 R(*Bln* I)	CCTAGGTTACTTTTTAAGCAGCTGCCG
pUC57-5 F(*Nco* I)	CATGCCATGGATGGCGATCTTTCTGACCAA
pUC57-5 R(*Hin*d III)	CCCAAGCTTTTATTTCTTCAGCAGCTGGC
pAD4-0067 F	GTGAGCGGATAACAATTTCACACAGGAAACAGACCATGGATGGGTGAATTTATCCGCTT
pAD4-0067 R	CCGGTACAGATCAAAATCAGACATTAATATATACCTCTTTTATTCTTTTTTATTTTTGC
pAD4-1647 F	AAAGAGGTATATATTAATGTCTGATTTTGATCTGTACC
pAD4-1647 R	GCTGAAAATCTTCTCTCATCCGCCAAAACAGCCAAGCTTTTACTTTTTAAGCAGCTGCC
up-parcA F	CCTGACTGTACTAACGGTT
up-parcA R	GCTAAAAAGCGCCGTTTTTTTTGACGGTGGTAAAGCCGAGTTTGCTACCTAAATTGCCA
down-parcA F	TCGGCTTTACCACCGTCAAA
down-parcA R	CTGACTTTTATGGCGTTC
up-pldhA F	CAAGCAGAATCAAGTTCTAC
up-pldhA R	TCTGAATCAGCTCCCCTGGGTTGCAGGGGAGCGGCAAGAAAGACTTTC

	TCCAGTGATG
down-pldhA F	TCTTGCCGCTCCCCTGCA
down-pldhA R	TGTCTGTTTCGCGGTCGCCA
up-patoB F	TACATAAAACGCCAATGG
up-patoB R	AAACACCCGATAACTTTCGCTATCGGGTGTTTTTATTGATTTATATTCCT TCTGTTT
down-patoB F	TCAATAAAAACACCCGATAG
down-patoB R	CATCAGCCCGGATGGGCAAA
up-ppflB F	ATATGACCGCAAATGGTC
up-ppflB R	ATTGTACGCTTTTTACTGTACGATTTCAGTCAAATCTAAGTAACACCTAC CTTCTTAAG
down-ppflB F	TTAGATTTGACTGAAATCGT
down-ppflB R	ACAGGTATGAATGCCTTC
veri-pRSF-0875 F	ACTTTAATAAGGAGATATACCAT
veri-pRSF-0875 R	CCGACGTCAGCGATCGCGTG
veri-pAD-1 F	CCCCTGTAGAAATAATTTTG
veri-pAD-1 R	ATAAACAAATAGGGGTTCCG
veri-pAD-4 F	TCAGGCAGCCATCGGAAGCT
veri-pAD-4 R	TTGCTCTTGCCCGGCGTCAA
veri-pAD-6 F	CAATTCCCCTGTAGAAATAA
veri-pAD-6 R	AGAGAACATAGCGTTGCCTT
veri-pRSF F	TGTAGAAATAATTTTGTT
veri-pRSF R	CAGCGATCGCGTGGCCGG
veri-arcA F	CGGAATCTTCGCGACCAAAC

veri-arcA R	TGGCCCTGAATTTAAGCGGT
veri-ldhA F	TTTATCGATATTGATCCAGG
veri-ldhA R	CTTTGTGTGCATTACCCAAC
veri-atoB F	GCACTCGGTATCGCTTACCT
veri-atoB R	AAGCTTGGCTGGTGGTCAAC
veri-pflB F	CTTTGTGTTGTCTGCGGAGC
veri-pflB R	CACCCATTGCCACCCATTTG

Supplementary Table S3 Synthesized genes or DNA sequences

Genes	Sequence (5'-3')
Tfu-0875	GAATTCATGACCGATGTTTATATTCTGGATGCCGTTCGGACTCCTTTCGGGCGCTACG GTGGGGCTTTATCGGGAATCCGGCCTGATGATCTGGCGGCACATGTCTTGCGGGCTT TAGCGGAACGTAGTCCGGGGCTGGATCCAGCGGCGGTCGACGATGTTTTCTTCGGT GATGCGAACGGCGCCGGTGAGGATAATCGCAACGTCGCCCGCATGGCTGCCCTGCT GGCGGGATGGCCTACCTCTGTGCCGGGTGTAACTTTGAACCGGCTTTGCGGGTCCG GCATGGAGTCCGTGATCGCTGCGAACCGCGCTATTGCGGTCGGTGACGCTTCGCTG GCCGTTGCGGGTGGTGTTGAATCGATGTCTCGCGCGCCGTGGGTGCTGCCGAAACC TGCTCAAGGTTTTCCGACTGGCCACGAAACGCTGTATTCCACAACTTTAGGCTGGCG CATGGTTAACCCAGCCATGCCGGAACAATGGACGGTGTCTCTGGGAGAGTCCACCG AGCAGGTGGCGGCGACGTACGGTATTTCTCGCGCGGAACAGGACGCCTTCGCGCTG CGCTCTCACGAACGTGCGGCGCGCGCTTGGGCAGAAGGCGTCTTCGACGCCGAAAT CACCCAAATTCCTGGTGCGGAGCTGGAGCGTGACGAGAGCATTCGCGAAACGTCGG CCGAGAAGCTCGCGGCGCTGAAACCGGCGTTTCGTCCTGATGGTACGATCACTGCA GGCAACGCGTCTCCTCTTAACGACGGCGCCGCAGCATTGCTGATTGGCGATGCAGCC GCTGCTGAACGTGTTGGTCGCGAACCATTAGCCCGGATCGTGAGCCGTGGTGTTGC CGCCGTAGACCCGGATGTGTTCGGGATCGGTCCGGTACAGGCCGCTGAGATTGCCC TGCGCCGGGCTGGGATTGGTTGGGATGACCTGTCGGTTGTCGAACTGAACGAAGCG TTTGCGGCCCAGAGCCTTGCGTGCCTGAAACTTTGGCCCGACCTGGATCCAGAAATT GTTAATCCTAACGGAGGCGCCATTGCGATTGGACATCCATTGGGCGCTAGTGGTGCC CGCATTGTTGGAACACTGGCTCACGAACTGCATCGGCGGGGTGGTGGCTGGGGCTT AGCTGCCATTTGTATCGGCGTCGGACAGGGGTTGGCCGTCGTTCTGCATCGTTAAAA GCTT
Tfu-2399	AGATCTATGGTTGAGGAAATTAATAAAGTGGGCGTAGTCGGCCTGGGTACAATGGG AGCGGGCATTGTCGAGGTATTTGCACGCGCCGGTTTCACCGTGACTGGCGTCGAAA TCGACGACGCGGCGCTGGAACGTGGACGGACCCATTTAGAAAAAAGCCTGGCCAAA

	GCTGTGGCAAAAGGAAAGCTCACCGAAGATGAGCAGCGCGCGATCCTCGGCCGCGT GACCTTCACCACGTCCCGCGATGACCTGGCAGACGCACATCTGGCCGTGGAGGCAG TTCCGGAGCGGCTGGACATTAAACGTTCCGTTTTCGCGGACCTCGATCGCATCTTACC CCCCGCAGCCATTCTGGCAACGAATACGTCAAGCCTGTCGGTTACCGAAATTGCGGC CCTGACGTCGCGCCCCGGCAAAGTTATTGGTCTGCACTTCTTCAACCCCGCGCCCGTT ATGCGTTTAGTGGAGATCGTTACCACCGTTGTAACCGAACCTCATGTTCGTGAAACC GCAACCCAGGTTGTGACCCGTCTGGGCAAAACACCAGTGGCGGTTGGAGATCGTGC GGGCTTTGTTGCCAACGCGCTGTTAGTGCCATATCTGAATCATGCAGTCGCAGTTTA CGAACAGGGCCTGGCGACCCGCGAACAGATCGATGCGGCCATTACCTCGGCAGCCG GTTTCCCGATGGGTCCTCTGACATTGATGGATCTGGTGGGTCTGGATGTTCTGTTGG ATGTTATGGATGTGTTATGGGATGAGTTCCGGCGCCCGCGTTATGCTGCAGCCCCAC TGCTGCGTCGCATGGTTGCTGCCGGGCTGCTTGGCCGCAAAAGTGGGCGCGGTTTTT ACGATTATTCCGGAGCCGACAACCCGGCAGAACCTGAACCTACGGCTCCTTTAGCAC AACTGGTTGGTGACGGGCCTGGCCAGATTTCGCTGGCGGACCTGCTGTTAGTTCCGC ATTTGAATGACGCAGCACGCATGATTGGCGATGGCTATGCTACCGCAGACGATGTA GATACGGCTATGCGTCTGGGCTGCGGGTATCCGAAAGGTTTAGCAGCCATGCTGGA TGAACGTGGAGTGAAAAACGTGACGGAGACCTTGGCAGAATTGGCTGCAGCCGGTT TGTTCACCGATGATACAGCGCCACTCCTGACGATGCTCGCGAAACAAGGTAAGGAC ACTCTGCGGAGTTAAGGTACC
Tfu-0067	CCATGGATGGGTGAATTTATCCGCTTTGAGTCTGACGGACCAGTTCGCCATATCGTC CTGAACGCCCCGCAGCGGCTGAATGCCCTGGATCGTCCGATGCTGGCAGAGCTGGC GGAGGCGGTACGCGCAGTGGCCGCCGATGAGGAGGCACGTGCCCTGGTAGTTTCG GGCGCAGGACGCGCTTTTTGCGCTGGCGCGGATGTTACGAGCTTATTTGGCGACCC GACCCGGCCCCCAGCGGTTATTCGTGACGAGCTGAAACAAGTCTATGCGAGTTTCTT GTCTATTGCGGATCTGACAATCCCAACGATCGCGGCGGTGGGAGGCATTGCCGTCG GCGCCGGTGTGAATATTGCAATGGCCTGCGATATGGTAGTGGCGGGTCCGAAAGCC AAATTTGCGATCACCTTCGCCGAAATGGGACTGCATCCGGGCGGTGGTTGCTCCTGG

	TTTCTGACGCGCCGTATGGGAGGCCATCGCGCTCTCGCGACTCTGTTAGACGCGGAA CGCATCGATGCCGAGGAGGCGTTCCGTGCTGGCCTTGTGACTCGCCTCGTCGAGGA TCCTGTGGCAGAAGCTCTTGCGATGGCACATAGCTATGCGGAACGGGACCCAGGCC TGGTACGGGACATGAAACGTGCCGTCCGCATGGCTGAAACTGCCGATCTTGCTACC GTGCTTGAATTTGAGTCGTGGGCGCAGGCTTCAAGCGTAAACTCCCCGCGCTTCCAG GAGTTTTTAGCGGAGTTTGCGGCGCGCAAAAATAAAAAAGAATAAAAGCTT
Tfu-1647	CATATGATGTCTGATTTTGATCTGTACCGGCCGACTGAAGAACATGAGGCTTTGCGT GAAGCAATTCGGAGTGTGGCCGAAGACAAAATCGCGCCGCATGCTGCCGATGTAGA TGAACAAAGCCGCTTTCCTCAGGAGGCATACGAAGCTCTCCGCGCATCCGATTTTCA TGCCCCGCATGTCGCGGAGGAGTACGGCGGTGTCGGAGCAGATGCCTTGGCGACCT GCATCGTCATTGAAGAAATCGCCCGGGTGTGTGCAAGCTCGTCACTGATCCCGGCCG TTAATAAACTGGGTAGTATGCCTTTGATTTTAAGCGGCTCAGACGAAGTGAAACAAC GTTATTTGCCCGAGCTTGCATCCGGGGAAGCGATGTTTAGTTATGGATTAAGTGAGC GCGAAGCCGGTTCGGACACGGCTTCAATGCGTACTCGCGCGGTCCGTGATGGCGAC GATTGGATCCTGAACGGTCAGAAATCGTGGATTACAAACGCGGGCATTTCAAAATAT TATACGGTCATGGCGGTCACCGACCCGGACGGCCCGCGTGGCCGCAACATTAGTGC ATTTGTTGTTCATATTGATGACCCTGGCTTCTCTTTTGGTGAGCCAGAGCGTAAATTA GGTATCAAAGGATCACCGACCCGCGAACTGATTTTTGACAACGTGCGTATCCCAGGT GATCGGCTTGTTGGTAAGGTTGGCGAAGGACTGCGTACCGCTCTGCGCACACTGGA CCATACCCGCGTCACCATCGGTGCACAGGCCGTTGGTATTGCTCAGGGTGCGCTTGA CTACGCATTAGGCTATGTTAAAGAACGTAAACAATTCGGTAAAGCGATTGCTGATTT TCAGGGAATTCAGTTCATGTTAGCTGACATGGCGATGAAGCTGGAAGCAGCACGTC AGATGGTTTATGTCGCAGCGGCAAAATCTGAACGCGATGATGCCGATCTGTCCTTCT ATGGCGCGGCAGCAAAGTGCTTCGCCAGTGATGTCGCTATGGAGATTACTACTGAT GCCGTGCAACTGCTGGGTGGTTATGGTTACACCCGGGACTATCCTGTTGAGCGCATG ATGCGCGACGCGAAAATCACACAAATCTACGAAGGTACGAACCAAATCCAGCGCGT TGTCATGGCACGGCAGCTGCTTAAAAAGTAACCTAGG

Tfu_2576-7	CCATGGATGGCGATCTTTCTGACCAAAGATTCTAAGGTGCTGGTGCAGGGCATGACC GGCAGTGAAGGGACCAAGCACACGCGCCGCATGCTGGCTGCAGGAACTAATATTGT TGGGGGTGTCAACCCACGCAAAGCTGGCCAAGTTGTAGATTTTGACGGGACGCAGG TGCCAGTATTTGGTAGTGTCGCGGAAGGCATGAAAGCTACGGGAGCGGATGTGACA GTCATTTTTGTTCCCCCAAAATTTGCGAAAGACGCGGTGATTGAGGCCATTGACGCT GAAATTGGCCTGGCAGTTGTTATTACCGAAGGAATCCCGGTACACGACACTGCTACG TTCTGGGCGCATGCGTGCTCTAAAGGAAATAAAACGCGTATCATCGGGCCAAATTGC CCGGGTTTAATTACACCGGGGCAGAGCAACGCTGGCATTATTCCTGCAGATATTACC AAACCCGGCCGCATCGGCCTGGTGAGCAAATCGGGGACGTTAACTTACCAGATGAT GTATGAATTACGTGACATCGGATTTAGTACCTGTGTGGGTATTGGCGGGGATCCGAT TATCGGTACCACGCATATTGATGCACTCGCCGCATTTGAAGCGGATCCGGATACGGA CGTTATCGTGATGATTGGAGAAATTGGCGGCGACGCGGAAGAGCGCGCAGCGGAG TACATCAAAAAACATGTTACCAAACCGGTGGTGGGGTATATTGCGGGGTTTACAGCA CCGGAAGGTAAAACGATGGGTCACGCCGGAGCGATCGTCTCCGGCTCTTCTGGGAC GGCCGCCGCAAAAAAAGAAGCCCTGGAGGCAGTCGGCGTGAAGGTGGGTAAAACT CCGAGTGAAGCTGCGAAGTTAGTCCGTTCGCTGTTCATGAGCGATTTCGATCTGTAC CGGCCAACCGAAGAACATGAAGCGTTACGCGAAGCTATCCGCTCAGTGGCCGAAGA TAAAATCGCACCACATGCCGCAGACGTGGATGAACAGAGCCGCTTCCCGCAGGAAG CGTACGAAGCACTTCGCGCATCAGATTTCCATGCTCCTCATGTTGCGGAAGAATATG GAGGCGTAGGCGCCGACGCCCTGGCAACGTGTATTGTGATCGAAGAAATTGCCCGT GTTTGTGCATCAAGCTCCCTCATCCCTGCGGTGAATAAGCTGGGCTCAATGCCCCTG ATTCTGTCGGGTTCTGATGAAGTGAAACAGCGGTACTTACCGGAACTGGCCTCTGGT GAGGCGATGTTTAGTTATGGCCTGAGCGAACGTGAAGCCGGCAGTGACACTGCTTC GATGCGTACGCGTGCTGTACGCGACGGTGACGATTGGATTCTGAACGGCCAGAAAA GTTGGATCACGAACGCGGGTATTAGCAAATACTACACCGTGATGGCGGTTACGGAT CCGGATGGCCCGCGCGGTCGTAACATCTCCGCATTTGTTGTACACATTGATGACCCT GGATTTTCGTTCGGCGAACCGGAACGTAAATTAGGGATCAAAGGCTCGCCGACGCG

	TGAATTGATCTTCGACAATGTTCGCATTCCCGGAGACCGTCTGGTGGGGAAAGTAG GTGAGGGCCTGCGTACAGCTCTGCGTACCCTTGACCATACTCGCGTGACGATTGGTG CGCAAGCCGTTGGGATCGCGCAAGGTGCACTGGATTATGCGCTTGGGTATGTTAAA GAACGCAAACAGTTTGGAAAAGCGATTGCCGATTTCCAGGGCATCCAATTTATGCTG GCCGACATGGCCATGAAACTGGAGGCGGCTCGCCAGATGGTCTACGTGGCTGCAGC GAAATCGGAGCGCGACGATGCGGATCTGAGCTTCTATGGCGCGGCGGCCAAATGTT TTGCCTCAGATGTGGCGATGGAAATTACCACGGATGCAGTCCAACTGTTAGGCGGTT ACGGCTATACGCGCGACTACCCCGTTGAACGTATGATGCGGGACGCGAAAATCACC CAGATTTACGAAGGTACCAATCAAATCCAACGCGTGGTGATGGCGCGCCAGCTGCT GAAGAAATAAAAGCTT
The sgRNA targeting *arcA* gene	TGACATCAACCTGGTGATCATGG
The sgRNA targeting *atoB* gene	AACGTGTTACAAGCCGGGCTGGG
The sgRNA targeting *ldhA* gene	TATATCGCCCTGCGCTGTGCCGG
The sgRNA targeting *pflB* gene	GAAAAACTACACTCCGTACGAGG
***The arcA* gene upstream and downstream 500bp from *E.coli* BL21**	CCTGACTGTACTAACGGTTGAATTGTTAAAAAATGCTACATATCCTTCTGTTTACTTA GGATAATTTTATAAAAAATAAATCTCGACAATTGGATTCACCACGTTTATTAGTTGTA TGATGCAACTAGTTGGATTATTAAAATAATGTGACGAAAGCTAGCATTTAGATACGA TGATTTCATCAAACTGTTAACGTGCTACAATTGAACTTGATATATGTCAACGAAGCGT AGTTTTATTGGGTGTCCGGCCCCTCTTAGCCTGTTATGTTGCTGTTAAAATGGTTAGG

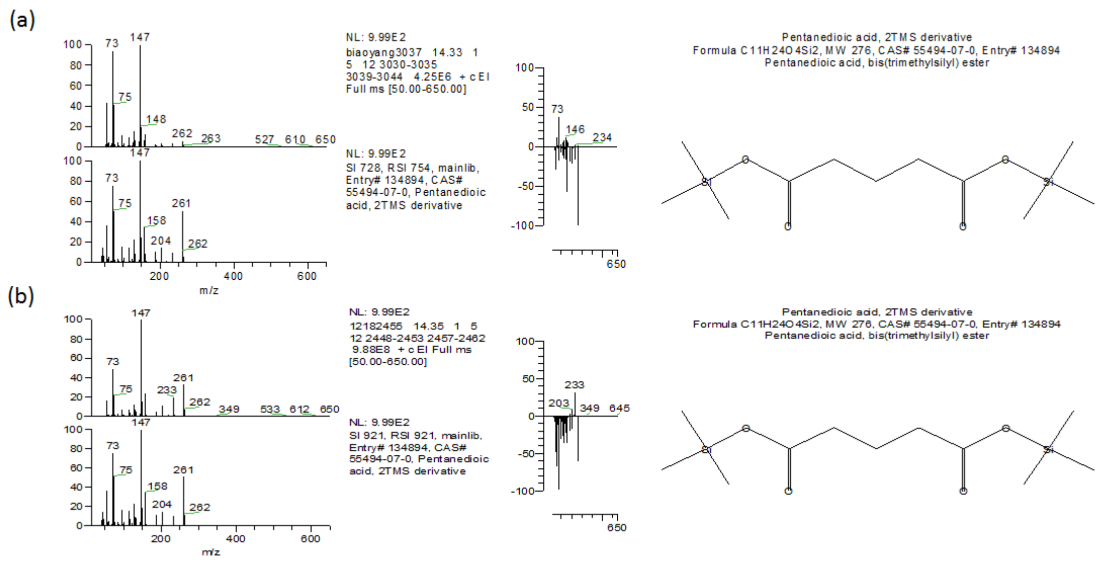
	ATGACAGCCGTTTTTGACACTGTCGGGTCCTGAGGGAAAGTACCCACGACCAAGCTA ATGATGTTGTTGACGTTGATGGAAAGTGCATCAAGAACGCAATTACGTACTTTAGTC ATGTTACGCCGATCATGTTAATTTGCAGCATGCATCAGGCAGGTCAGGGACTTTTGT ACTTCCTGTTTCGATTTAGTTGGCAATTTAGGTAGCAAACTCGGCTTTACCACCGTCA AAAAAAACGGCGCTTTTTAGCGCCGTTTTTATTTTTCAACCTTATTTCCAGATACGTAA TTGATCGTCCATTGTAACTTCTTTACTGGCTTTCATTTTCGGCAGTGAAAACGCATACC AGTCGATATTACGAGTCACAAACATCATGCCGGCCAGCGCCACCACCAGCACACTGG TTCCCAACAACAGCGCGCTATCGGCAGAGTTGAGCAGTCCCCACATCACACCATCCA GCAACAACAGCGCGAGGGTAAACAACATGCTGTTGCGCCAGCCTTTCAATACCGCCT GCAAATAAATACCGTTCATTAACGCCCCAATCAGACTGGCGATTATCCATGCCACGG TAAAACCGATATGTTCAGAAAGCGCCAGCAAGAGCAAATAAAACATCACCAATGAA AGCCCCACCAGCAAATATTGCATTGGGTGTAAACGTTGCGCGGTGAGCGTTTCAAAA ACAAAGAACGCCATAAAAGTCAG
The *ldhA* gene upstream and downstream 500 bp from *E. coli* BL21	CAAGCAGAATCAAGTTCTACCATGCCGACGTTCAATAACCAGCGGCTGGGATGTGA AAGGCTGGCGTTGGTGATATGCGCAAGCTGACAATCTCCCACCAGATAACGGAGAT CGGGAATGATTAAACCTTTACGCGTAATGCGTGGGCTTTCATCTAATGCAATACGTG TCCCGAGCGGTAGCCAGATGCCCGCCAGCGTGGGAACCCACAGCCCGAGCGTCATC AGCAGCGTCAACGGCACAAGAATAATCAGTAATAACAGCGCGAGAACGGCTTTATA TTTACCCAGCATGGGTAGTTAATATCCTGATTTAGCGAAAAATTAAGCATTCAATACG GGTATTGTGGCATGTTTAACCGTTCAGTTGAAGGTTGCGCCTACACTAAGCATAGTT GTTGATGAATTTTTCAATATCGCCATAGCTTTCAATTATATTTGAAATTTTGTAAAATA TTTTTAGTAGCTTAAATGTGATTCAACATCACTGGAGAAAGTCTTTCTTGCCGCTCCC CTGCAACCCAGGGGAGCTGATTCAGATAATCCCCAATGACCTTTCATTCTCTATTCTT AAAATAGTCCTGAGTCAGAAACTGTAATTGAGAACCACAATGAAGAAAGTAGCCGC GTTTGTTGCGCTAAGCCTGCTGATGGCGGGATGTGTAAGTAATGACAAAATTGCTGT TACGCCAGAACAGCTACAGCATCATCGCTTTGTGCTGGAAAGCGTAAACGGTAAGCC CGTGACCAGCGATAAAAATCCGCCAGAAATCAGCTTTGGTGAAAAAAATGATGATTTC

	CGGCAGCATGTGTAACCGCTTTAGCGGTGAAGGCAAACTGTCTAATGGTGAACTGA CAGCCAAAGGGCTGGCAATGACCCGTATGATGTGCGCTAACCCGCAGCTTAATGAA CTCGATAACACCATTAGCGAAATGCTGAAAGAAGGTGCACAAGTGGATCTGACCGC GAACCAGTTAACGCTGGCGACCGCGAAACAGACA
The *atoB* gene upstream and downstream 500 bp from *E. coli* BL21	TACATAAAACGCCAATGGCTTATATGCGTGCTATCAGCGCGGCAGCACGCAGTACTG CCGGTATTCTGGTGCAATTCCCCTTCTACGCTGGGATCCAACTGATGATGGAGCATTC CGGTCTGGGCGGACTCATTACCGAATTCTTCATCAATGTTGCGAACAAAGACACCTT CCCGGTAATGACCTTTTTTAGTTCTGCACTGATTAACTTCGCCGTTCCGTCTGGCGGC GGTCACTGGGTTATTCAGGGACCTTTCGTGATACCCGCAGCCCAGGCGCTGGGCGCT GATCTCGGTAAATCGGTAATGGCGATCGCCTACGGCGAGCAATGGATGAACATGGC ACAACCATTCTGGGCGCTGCCAGCACTGGCAATCGCCGGACTCGGTGTCCGCGACAT CATGGGCTACTGCATCACTGCCCTGCTCTTCTCCGGTGTCATTTTCGTCATTGGTTTAA CGCTGTTCTGACGGCACCCCTACAAACAGAAGGAATATAAATCAATAAAAACACCCG ATAGCGAAAGTTATCGGGTGTTTTCTTGAACATCGACGGCGAAGGTAACCCCATTAA TCACCAGTCAAAACTTTTCACCAGCGTCAGCTCACCAGCATTACGCATCGGTACAATA AATGTTTCCTGTTTCTCATTGACCGATCCTTCATCGGTGATCAGCGTCAGTTGGGCGG TGGTTAATTCCGTTTCGCTGCGCCCACCATAGTAGTTGATATACACCTGATAGCGCCC GTGAATTGGCGCGGGCATGGCGAAAATCTCGGGTCCGTACCCCGTCGTGACATCCA TATCCAGTGCACCACTGTTTTTCAGCACGGTGTTACCGTACCAGGCGTGTTCGCCATC GGGCGTAACAACGTGAAGGTCGAGGTCGGTATTGTCCGTATCCCACGAGAGAACCA GCCGTAAACGTGCACGAATCGTTCCTGTACCCGGCGTTGAGTAAAACTGCATTTTTT GTCGGCTTTGCCCATCCGGGCTGATG
***The pflB* gene upstream and downstream 500bp from *E.coli* BL21**	ATATGACCGCAAATGGTCAATGGGGACTAAACGTCCTACAAACCGCCGACCACAAA GTGCACCATACTTTTATTGAGGCCGTCTGTCTTGGTATCCTGGCAAACCTGATGGTAT GTCTGGCAGTATGGATGAGTTATTCTGGCCGCAGCCTGATGGACAAAGCGTTCATTA TGGTGCTGCCGGTCGCGATGTTTGTTGCCAGCGGTTTTGAGCACAGTATCGCAAACA TGTTTATGATCCCGATGGGTATTGTAATCCGCGACTTCGCATCCCCGGAATTTTGGAC

	CGCAGTCGGTTCTGCACCGGAAAATTTTTCTCACCTGACCGTGATGAATTTCATCACT GATAACCTGATTCCGGTTACGATCGGCAACATTATCGGTGGTGGTTTGTTGGTTGGG TTGACATACTGGGTCATTTACCTGCGTGAAAACGACCACCATTAATGGTTGTCGAAG TACGCAGTAAATAAAAAATCCACTTAAGAAGGTAGGTGTTACTTAGATTTGACTGAA ATCGTACAGTAAAAAGCGTACAATAAAGGCTCCACGAAAGTGGGGCCTTTTTTAGCG CGAGAGCCTTTTTTGTCAGCTATCTATACTTTAAGGTGACTGCCAAAACAGACTCGAC GTAGCCTTCGAGCTGCGCACCAACACGGCCTCAGATGGGCCACATCTGGAGAAACA CCGCAATGTCAGTTATTGGTCGCATTCACTCCTTTGAATCCTGTGGAACCGTAGACG GCCCGGGTATTCGCTTTATCACCTTTTTCCAGGGCTGCCTGATGCGCTGCCTGTATTG TCATAACCGCGACACCTGGGACACGCATGGCGGTAAAGAAGTTACCGTTGAAGATT TGATGAAGGAAGTGGTGACCTATCGCCACTTTATGAACGCTTCCGGCGGCGGCGTT ACCGCATCCGGCGGTGAAGCAATCCTGCAAGCTGAGTTTGTTCGTGACTGGTTCCGC GCCTGCAAAAAAGAAGGCATTCATACCTGT

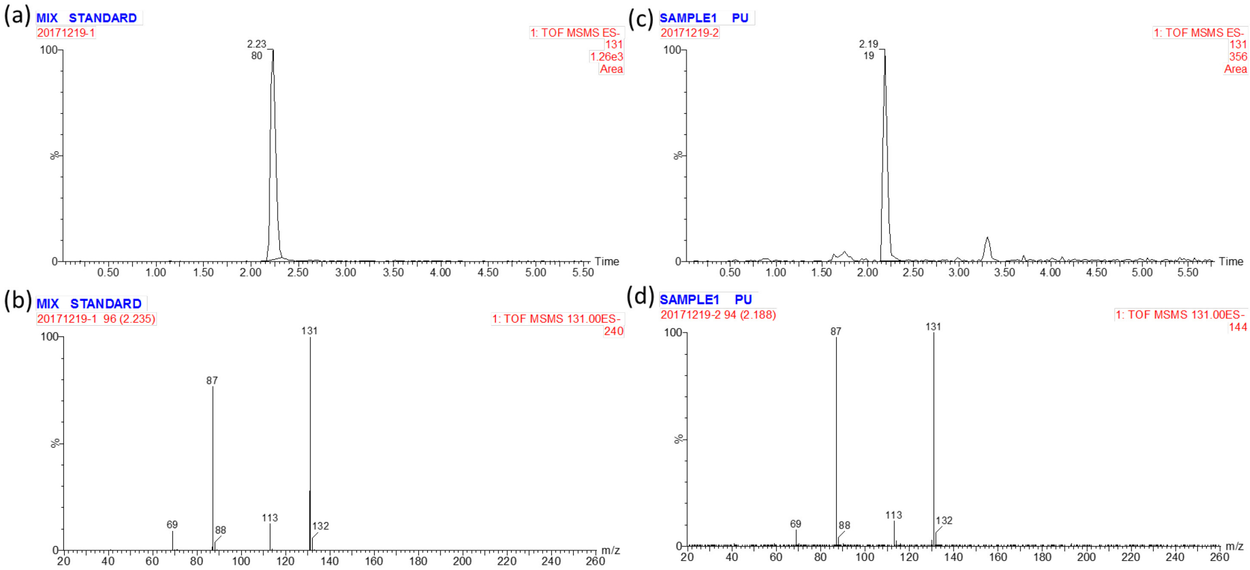
Supplementary Table S4 Gradient elution program for LCMS-Q-TOF

Time (min)	Buffer A (%)	Buffer B (%)
0.0	0	100
0.1	0	100
5.0	60	40
7.0	80	20
8.0	100	0
8.1	0	100
10.0	0	100



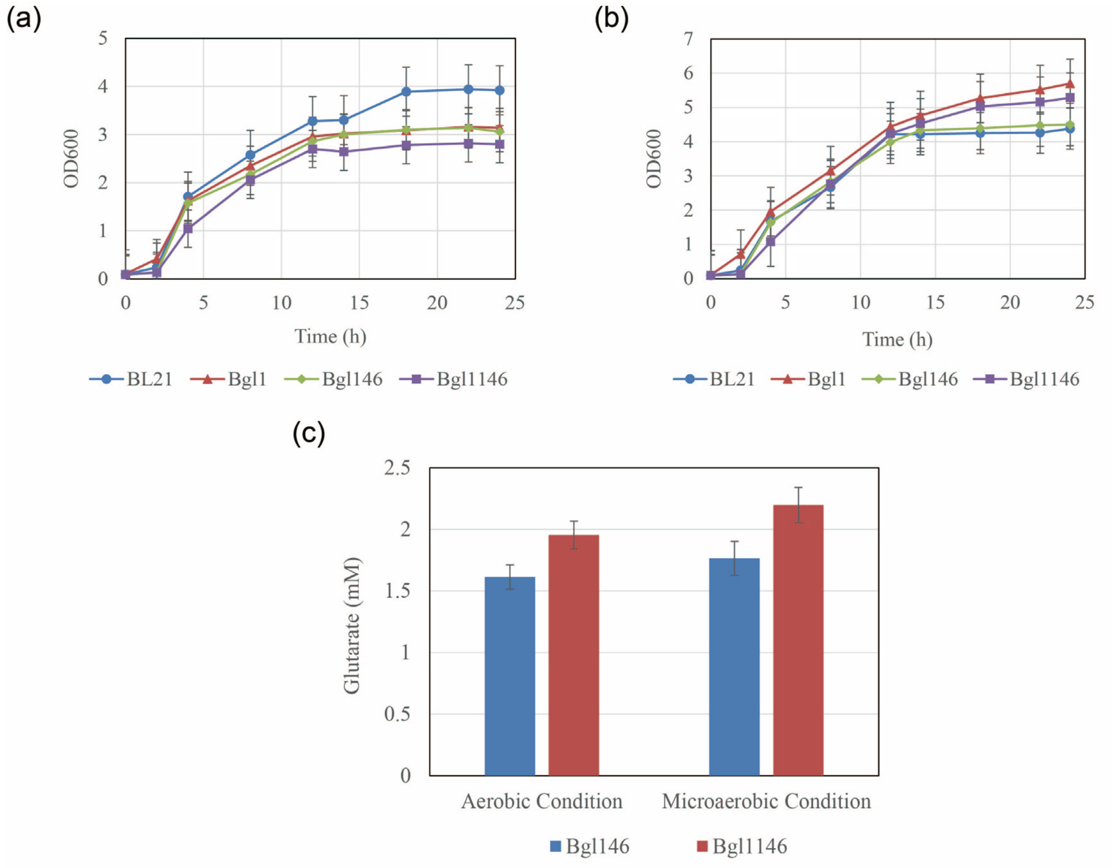
Supplementary Figure S1. Analysis of glutarate by GC-MS.

(a) Glutarate standard; (b) Glutarate from fermentation broth.



Supplementary Figure S2. LCMS-Q-TOF results.

(a) UPLC result of glutarate standard; (b) Mass spectrum of glutarate standard; (c) UPLC result of glutarate sample; (d) Mass spectrum of glutarate sample.



Supplementary Figure S3. The effect of *arcA* knockout on the fermentation of glutarate.

The engineered strains *E. coli* BL21, Bgl1 (BL21Δ*arcA*), Bgl146 (BL21 carrying pAD1, pAD4 and pAD6), and Bgl1146 (Bgl1 carrying pAD1, pAD4 and pAD6) were cultured on 22.22 mM glucose in aerobic condition and microaerobic condition, (a) The growth curve of each engineered strain in the microaerobic condition; (b) The growth curve of each engineered strain in the aerobic condition; (c) Fermentation results for engineered strain *E. coli* Bgl146 and Bgl1146 in shaken flasks in aerobic condition and microaerobic condition.

Supplementary Materials and Methods

Media components

The media were prepared as follow(3):

Lysogeny broth (LB): 10 g L^{-1} NaCl, 10 g L^{-1} tryptone, and 5 g L^{-1} yeast extract. For agar plates.

Terrific broth (TB): 12 g L^{-1} tryptone, 24 g L^{-1} yeast extract, 4 mL glycerol, and phosphate buffer prepared as a 10× stock solution (final concentration in medium 6.3 g L^{-1} K_2HPO_4 and 1.3 g L^{-1} KH_2PO_4).

Super optimal broth (SOB): 20 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 2.47 g L^{-1} $MgSO_4 \cdot 7H_2O$, 5 g L^{-1} NaCl, 0.186 g L^{-1} KCl, and supplemented with various concentrations of glucose.

Super optimal broth with catabolite repression (SOC): 20 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 2.47 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1} NaCl, 0.186 g L^{-1} KCl, 2.033 g L^{-1} $MgCl_2 \cdot 6\ H_2O$, and supplemented with various concentrations of glucose.

M9: 12.8 g L^{-1} Na_2HPO_4, 3 g L^{-1} KH_2PO_4, 0.5 g L^{-1} NH_4Cl, and 0.5 g L^{-1} NaCl. The 10 g L^{-1} glucose, 0.493 g L^{-1} $MgSO_4 \cdot 7H_2O$, and 0.15 g L^{-1} $CaCl_2 \cdot 2H_2O$ was sterilized separately.

MOPS minimal medium (MOPS): 40 mM MOPS (3-(N-morpholino) propanesulfonic acid) and 1.69 mM K_2HPO_4, supplemented with 22.2 mM glucose, 100 µM $FeSO_4$, 5 mM calcium pantothenate, 5 mM $(NH_4)_2SO_4$, and 30 mM NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM $CaCl_2 \cdot 2H_2O$, 9.6 mM glycine, and 1 mL trace-element stock solution per L medium(4). The trace-element stock solution (pH 7.5) contained 13.4 mM EDTA, 31 mM $FeCl_3$, 6 mM $ZnCl_2$, 1.6 mM H_3BO_3, 0.8 mM $CuCl_2 \cdot 2H_2O$, 0.4 mM $CoCl_2 \cdot 6H_2O$, and 3 mM $(NH_4)_6MO_7O_{24} \cdot 4H_2O$ and 0.08 mM $MnCl_2 \cdot 4H_2O$.

R medium: 2.5 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 2.5 g L^{-1} NaCl, 3 g L^{-1} cysteine, and supplemented with various concentrations of glucose.

Supplementary References

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4. Clomburg JM, Vick JE, Blankschien MD, Rodriguez-Moya M, Gonzalez R. 2012. A synthetic biology approach to engineer a functional reversal of the beta-oxidation cycle. ACS Synth Biol 1:541-554.