Supplementary Information for

Article

Hydroxybenzoic acid production using metabolically engineered *Corynebacterium glutamicum*

Misa Doke [1], Mayumi Kishida [1], Yuuki Hirata [1], Mariko Nakano [1], Mayo Horita [1], Daisuke Nonaka [1], Yutaro Mori [1], Ryosuke Fujiwara [2], Akihiko Kondo [2,3], Shuhei Noda [2,3,4], and Tsutomu Tanaka [1,*]

Table S1. Plasmids used in this study.

Plasmid	Genotype	Source or reference
pCCS	E. coli–C. glutamicum shuttle vector for control, KmR	Matsuura et al.
pCC-H36-hyg5	pCCS containing hyg5 under the control of the H36 promoter	This study
pCC-H36-ubiC	pCCS containing hyg5 under the control of the H36 promoter	This study
pCC-e10-hyg5	pCCS containing hyg5 under the control of the dap-e10 promoter	This study
pCC-e11-hyg5	pCCS containing hyg5 under the control of the dap-e11 promoter	This study
pCC-e12-hyg5	pCCS containing hyg5 under the control of the dap-e12 promoter	This study
pCC-tacM1-hyg5	pCCS containing hyg5 under the control of the tacM1 promoter	This study
pCC-J2-hyg5	pCCS containing hyg5 under the control of the J2 promoter	This study
pCC-J3-hyg5	pCCS containing hyg5 under the control of the J3 promoter	This study
pCC-J4-hyg5	pCCS containing hyg5 under the control of the J4 promoter	This study
pCC-e10-ubiC	pCCS containing ubiC under the control of the dap-e10 promoter	This study
pCC-e11-ubiC	pCCS containing ubiC under the control of the dap-e11 promoter	This study
pCC-e12-ubiC	pCCS containing ubiC under the control of the dap-e12 promoter	This study
pCC-tacM1-ubiC	pCCS containing ubiC under the control of the tacM1 promoter	This study
pCC-J2-ubiC	pCCS containing ubiC under the control of the J2 promoter	This study
pCC-J3-ubiC	pCCS containing ubiC under the control of the J3 promoter	This study
pCC-J4-ubiC	pCCS containing ubiC under the control of the J4 promoter	This study
pCC-e10-pchB	pCCS containing pchB under the control of the dap-e10 promoter	This study
pCC-e11-pchB	pCCS containing pchB under the control of the dap-e11 promoter	This study
pCC-e12-pchB	pCCS containing pchB under the control of the dap-e12 promoter	This study
pCC-tacM1-pchB	pCCS containing pchB under the control of the tacM1 promoter	This study
pCC-J2-pchB	pCCS containing pchB under the control of the J2 promoter	This study
pCC-J3-pchB	pCCS containing pchB under the control of the J3 promoter	This study
pCC-J4-pchB	pCCS containing pchB under the control of the J4 promoter	This study
pCC-e10-entC-pchB	pCCS containing entC-RBS-pchB under the control of the dap-e10 promoter	This study
pCC-e11-entC-pchB	pCCS containing entC-RBS-pchB under the control of the dap-e11 promoter	This study
pCC-e12-entC-pchB	pCCS containing entC-RBS-pchB under the control of the dap-e12 promoter	This study
pCC-tacM1-entC-pchB	pCCS containing entC-RBS-pchB under the control of the tacM1 promoter	This study
pCC-J2-entC-pchB	pCCS containing entC-RBS-pchB under the control of the J2 promoter	This study

pCC-J3-entC-pchB	pCCS containing entC-RBS-pchB under the control of the J3 promoter	This study
pCC-J4-entC-pchB	pCCS containing entC-RBS-pchB under the control of the J4 promoter	This study
pCC-e10-pchB-entC	pCCS containing pchB-RBS-entC under the control of the dap-e10 promoter	This study
pCC-e11-pchB-entC	pCCS containing pchB-RBS-entC under the control of the dap-e11 promoter	This study
pCC-e12-pchB-entC	pCCS containing pchB-RBS-entC under the control of the dap-e12 promoter	This study
pCC-tacM1-pchB-entC	pCCS containing pchB-RBS-entC under the control of the tacM1 promoter	This study
pCC-J2-pchB-entC	pCCS containing pchB-RBS-entC under the control of the J2 promoter	This study
pCC-J3-pchB-entC	pCCS containing pchB-RBS-entC under the control of the J3 promoter	This study
pCC-J4-pchB-entC	pCCS containing pchB-RBS-entC under the control of the J4 promoter	This study
pK18mobsacB	sacB, lacZ, KmR, MCS, mobilizable vector, enables selection/counter-selection for integration/excision in C. glutamicum	ATCC
pK18-ΔpheA	pK18mobsacB derivative for pheA deletion	This study
pK18-ΔtyrA	pK18mobsacB derivative for tyrA deletion	This study
pK18-Δcg0975	pK18mobsacB derivative for cg0975 deletion	This study
pK18-Δcg0344-47	pK18mobsacB derivative for cg0344-cg0347 deletion	This study
pK18-Δcg3349-54	pK18mobsacB derivative for cg3349-cg3354 deletion	This study
pK18-Δpqo	pK18mobsacB derivative for pqo deletion	This study
pK18-Δldh	pK18mobsacB derivative for ldh deletion	This study
pK18-Δlcg2966	pK18mobsacB derivative for cg2966 deletion	This study

Table S2. Primers used in this study.

name	**Sequence(5' → 3')**
EcoRI_pheA_UP_for	ACATGATTACGAATTCCGGTGGATTCGAGGATGCTCAGGAAG
pheA_down_BamHI_re	CGACTCTAGAGGATCCGAGTAATTCATGTGGGCTTCTTCATCGCCG
pheA-down_for	CGGGTTAAGCTGTGTAACGTCTTCGGAACCTACCGCTTCCACCTG
pheA_UP_re	CGGTAGGTTCCGAAGACGTTACACAGCTTAACCCGCCGAACTAAGGTG
EcoRI_cg-tyrA_UP_for	ACATGATTACGAATTCCGTAAATGACGGCGCCAACGGGAAC
cg-tyrA_down_BamHI_re	CGACTCTAGAGGATCCCCATTGCTGTCCTGGTGTTGTTCCTGAC
cg-tyrA-down_for	CCCGCCCCGGCTGATGATTCTCAGGCGATTCCTAATG
cg-tyrA_UP_re	CGCCTGAGAATCATCAGCCGGGGCGGGAAATGTCTTTGGTAGTCAC
EcoRI_cg-0975CM_UP_for	ACATGATTACGAATTCCCCTGGCCACGACTGTTTCATAAGGATTG
cg-0975CM_down_BamHI_re	CGACTCTAGAGGATCCAGCATCGACGGAGGCTCCGATTTCG
cg-0975CM-down_for	GCCAGACGTGGCAGCTGCGCATGGGACGCGGAAAACTC
cg-0975CM_UP_re	CGCCTGAGAATCATCAGCCGGGGCGGGAAATGTCTTTGGTAGTCAC
EcoRI_cg2624-40del_UP_for	ACATGATTACGAATTCAACCTCTAGTCCCTCAGGCAGGTAGCCG
cg2624-40del_down_BamHI_re	CGACTCTAGAGGATCCCCGGTGATGCGCTGGATGTCTGTG
cg2624-40del-down_for	GGGTTCGCGGAGGCGACCAGGGATAACCCTTGGTC
cg2624-40del_UP_re	CCCTGGTCGCCTCCGCGAACCCTTGTGCATATGATGAACATTACG
EcoRI_cg3349-54del_UP_for	ACATGATTACGAATTCCAGTGTCTGCCGTGATTGCGCGTTTG
cg3349-54del_down_BamHI_re	CGACTCTAGAGGATCCGCAATCGCAGTCATCGTGAACAAATTCG
cg3349-54del-down_for	GGCTCTGGAACCTCTCCGCCCTCCTCGAACAGAAATAGCAGCC
cg3349-54del_UP_re	CGAGGAGGGCGGAGAGGTTCCAGAGCCACCGCGCTG

EcoRI_cg0344-47del_UP_for	ACATGATTACGAATTCCCACAGGGTTTCGGGGGTGGTGATAATC
cg0344-47del_down_BamHI_re	CGACTCTAGAGGATCCGTCCTGTCCATCTGGACCATGTCCTGG
cg0344-47del-down_for	GTGTCTGGGATCACGCTACCTTCCTCACCCGCTTCTACGC
cg0344-47del_UP_re	CGGGTGAGGAAGGTAGCGTGATCCCAGACACTTCGTCGGGGC
EcoRI_cg-pobANotI_UP_for	ACATGATTACGAATTCGATGATGTTGAAGGACATCGTCTTTGCGAGG
cg-pobANotI_down_BamHI_re	CGACTCTAGAGGATCCCTCATCGGGTTCGAGGAAGAAATCGCC
cg-pobANotI-down_for	GGAGTTCCCGCGGCCGCTCCGCATCGAAGACTCTGAGGCATCG
cg-pobANotI_UP_re	CTTCGATGCGGAGCGGCCGCGGGAACTCCTTTCATTGACCCACTGGAG
EcoRI_cg2966Ph_UP_for	ACATGATTACGAATTCCTACCTTGAAATTGTTGGCTCAAAAGGCCCTGAG
cg2966Ph_down_BamHI_re	CGACTCTAGAGGATCCGAATAATTCCTTCCATCGCTCCCCACGTGC
cg2966Ph-down_for	GGAAATCCCCTCGGCGCACATCAGCTAATCAACGCGAACTAAG
cg2966Ph-A_UP_re	GATTAGCTGATGTGCGCCGAGGGGATTTCCTATCTCATTCACGGAGCG
dap-A16-1_Nhe_cgR0949_for	TGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCGAAAGGAT-TTCGCTAATGGCTAGCCAAATAAACCGCCGAGGCTTCTTAAAAGC
H36_dap-A16-1_re	GTACGAAAATTGCTTTCATTGTTGATCTCCTTTTTAAGTGAACTT-GGGCCCTTAGCATGCTACTCCTACCAACCAAGGTGCG
dap-e10_A16_for	AAATGAGGGAATGTGGTATAATTGAACTCG-GATCCCTAAGGGCCCAAGTTCACTTAAAAAGGAGATCAAC
dap-e10_re	TATACCACATTCCCTCATTTGGTGTCAAAACAACTCGACCAACAGTTGCG-CAGCCTG
dap-e11_Bam_bc-A16_for	CAAATGAGGGAATGTGCTATAATGGAACTCG-GATCCCTAAGGGCCCAAGTTCACTTAAAAAGGAGATCAAC
dap-e11_re	CCATTATAGCACATTCCCTCATTTGGTGTCAAAACAACTCGACCAACAG-TTGCGCAGCCTG
dap-e12_Bam_bc-A16_for	CCAAATGAGGGAATGTGGTAGAGTGGAACTCG-GATCCCTAAGGGCCCAAGTTCACTTAAAAAGGAGATCAAC
dap-e12_re	CTCTACCACATTCCCTCATTTGGTGTCAAAACAACTCGACCAACAGTT-GCGCAGCCTG
tac-M1_Bam_bc-A16_for	GTGTGCTATAATGGGTGGAATTGTGAGCGGATAACAATT-GGATCCCTAAGGGCCCAAGTTCACTTAAAAAGGAGATCAAC
tac-M1_re	CCACCCATTATAGCACACGATGATTAATTGTCAACAGCTCACTCGAC-CAACAGTTGCGCAGCCTG
J2_Bam_bc-A16_for	TAGTTTTGAGTTACAATGGTTGGGATCCCTAAGGGCCCAAGTTCAC-TTAAAAAGGAGATCAAC
J2_re	CCCATTGTAACTCAAAACTAAAAAAATGTCAATCGACCAACAGTTGCG-CAGCCTG
J3_Bam_bc-A16_for	AAGGTTGTATGTGCTATAATGGACCGGATCCCTAAGGGCCCAAGTTCAC-TTAAAAAGGAGATCAAC
J3_re	AGCACATACAACCTTATTTGTCAACTCGACCAACAGTTGCGCAGCCTG
J4_Bam_bc-A16_for	TTTGAATCTGTGTTATAATGGTTCGGATCCCTAAGGGCCCAAGTTCAC-TTAAAAAGGAGATCAAC
J4_re	TAACACAGATTCAAATTAATGTCAACTCGACCAACAGTTGCGCAGCCTG
H36_BamHI_Hyg5_for	GAGTAGCATGGGATCCATGCTGAACCCATCCTCCTTGGTGCTGAACGGCC
Hyg5_XhoI_re	ACAGCCAAGCCTCGAGTTACATGACCACGCCTTCGATTTCCACGAG-CAGATC
H36_BamHI_UbiC_for	GAGTAGCATGGGATCCATGTCTCACCCAGCACTGACCCAGCTTC
UbiC_XhoI_re	ACAGCCAAGCCTCGAGTTAGTACAGTGGAGATGCTGGCAGGAACAGTTC

NheI_Hyg5_for	TTCGCTAATGGCTAGCCTGAACCCATCCTCCTTGGTGCTGAACG
XhoI_Hyg5_re	ACAGCCAAGCCTCGAGTTACATGACCACGCCTTCGATTTCCACG
NheI_UbiC_for	TTCGCTAATGGCTAGCTCTCACCCAGCACTGACCCAGCTTCG
XhoI_UbiC_re	ACAGCCAAGCCTCGAGTTAGTACAGTGGAGATGCTGGCAGGAACAG-TTCG
NheI_pchB_for	TTCGCTAATGGCTAGCAAAACCCCAGAAGATTGCACCGGTC
XhoI_pchB_re	ACAGCCAAGCCTCGAGTTATGCTGCGCCACGGGTCTGACG
NheI_entC_for	TTCGCTAATGGCTAGCTCTGCCCACCGCAACCCCTCTG
XhoI_entC_re	ACAGCCAAGCCTCGAGTCACAAACCAAGCGAGCGCATGATG
Xho_entC_RBS_pchB_for	CTTGGTTTGTGACTCGAGGA-GAAAGGAGGCCCTTCAGATGAAAACCCCAGAAGATTGCACCGGTC
Xho_pchB_RBS_entC_for	CTTGGTTTGTGACTCGAGGA-GAAAGGAGGCCCTTCAGATGTCTGCCCAC-CGCAACCCCTCTG

A sequence of codon-optimized genes

Hyg5

ATGCTGAACCCATCCTCCTTGGTGCTGAACGGCCTGACCTCCTACTTCGAAAACGGTCGCG-
CACGCGTGGTGCCACCAGTGGGTCGCAACATCCTCGGCGTGGTGAACTACGCATCCGTGTGCGAATACCCAACCTTGGAT
CACGGCTACCCAGAACTGGAAATCAACATGGTGGCTCCAACCGCAGAAC-
CATTCGCCGAAGTGTGGGTGACCGATGCAGAATCCGAACACGGCGAACGCGACGGCATCACCTACGCACACGATGGCGA
ATACTTTTTCTGCGCAGGTCGCGTGCCACCAACCGGTCGCTACACCGAAGCAACCCGTGCAG-
CATACGTGACCATGTTCGAACTGCTGGAAGAGTTCGGCTACTCCTCCGTGTTCCGCATGTGGAACTTCATCGGCGATATCA
ACCGCGATAACGCCGAAGGCATGGAAGTGTACCGCGATTTCTGCCGTGGTCGCGCTGAA-
GCATTCGAACAGTGCCGCTTGGAGTTCGATCAGTTCCCAGCAGCAACCGGCATCGGCTCCCGTGGCGGTGGTATCGCATTC
TACCTGCTGGCATGCCGCTCCGGTGGCCACGTGCACATCGAAAACCCACGCCAGGTGCCAG-
CATACCACTATCCAAAGCGCTACGGTCCACGCGCACCACGCTTCGCACGCGCAACCTACCTGCCATCTCGCGCAGCAGAT
GGCGTCGGTGGCCAGGTGTTCGTGTCCGGCACCGCATCCGTCCTGGGCCACGAAAC-
CGCTCACGAAGGCGATCTGGTGAAGCAGTGCCGTCTGGCACTGGAAAACATCGAACTGGTGATCTCCGGTGGTAACCTGG
CAGCACACGGCATCTCCGCAGGCCACGGTCTGACCGCACTGCGCAACATCAAGGTGTAC-
GTGCGTCGCTCCGAAGATGTGCCAGCAGTGCGCGAAATCTGTCGCGAAGCATTCTCCCCAGATGCAGATATCGTGTACCTG
ACCGTGGATGTGTGCCGTTCCGATCTGCTCGTGGAAATCGAAGGCGTGGTCATGTAA

UbiC

ATGTCTCACCCAGCACTGACCCAGCTTCGCGCACTGCGCTACTGCAAAGAAATCCCAGCAC-
TCGATCCACAGCTGCTGGACTGGCTGCTGCTGGAAGATTCCATGACCAAGCGCTTCGAACAGCAGGGCAAGACCGTGTCC
GTGACCATGATCCGCGAAGGCTTCGTGGAACAGAACGAGATCCCAGAAGAACTGCCAC-
TGCTGCCAAAAGAATCCCGCTACTGGCTGCGCGAAATCCTGCTGTGCGCAGATGGCGAACCATGGCTGGCAGGTCGCACC
GTGGTGCCAGTGTCCACCTTGTCCGGTCCAGAACTGGCCCTGCAGAAGCTCGGCAA-
GACCCCACTGGGTCGCTACCTGTTCACCTCCTCCACCTTGACTCGCGATTTCATCGAGATCGGTCGCGACGCAGGCCTGTG
GGGTCGTCGCTCCCGTCTGCGCCTGTCCGGCAAGCCACTGCTCCTGAC-
CGAACTGTTCCTGCCAGCATCTCCACTGTACTAA

pchB

ATGAAAACCCCAGAAGATTGCACCGGTCTGGCAGATATCCGCGAAGCAATCGATCG-
CATCGATCTGGATATCGTGCAGGCACTGGGTCGCCGTATGGATTACGTGAAGGCAGCATCCCGCTTCAAGGCATCCGAAG
CAGCAATCCCAGCACCAGAACGCGTGGCTGCAATGCTGCCTGAACGCGCACGCTGGGCTGAA-
GAAAACGGCCTGGATGCACCATTCGTGGAAGGCCTGTTCGCACAGATCATCCACTGGTACATTGCAGAGCAGATCAAGTA
CTGGCGTCAGACCCGTGGCGCAGCATAA