Supplemental materials to:

High-yield production of 4-hydroxybenzoate from glucose or glycerol by an engineered *Pseudomonas taiwanensis* VLB120

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**Codon-optimized genes**

**Table S1: Oligonucleotides used in this study.** Restriction sites are underlined.

|  |  |  |
| --- | --- | --- |
| **Primer** | **5’ – Sequence – 3’** | **Description** |
| CL160 | GGCCGCGGCCGCGCGAATTCAGGAGGTACCAGCTATGAGCAAATATGAAGGCCGC | Cloning pBELK ferulic s:Fwd *ech*- *vdh*-*fcs*, *EcoRI* |
| CL161 | CAGGTCGACTCTAGAGGATCCCGATGGCCGTGTCCTCGA | Cloning pBELK ferulic s:Rev *ech*-*vdh*-*fcs*, BamHI |
| CL166 | CAATTTCACAGGTACCGAATTCAGGAGGTACCAGCTATGGCACCCTCGCTCGAC | Cloning pJT’ *Rt*PAL:Fwd *Rt*PAL, *EcoRI* |
| CL167 | ACGTCGCATGCTCCTCTAGATTCTAAGCGAGCATCTTGAGGAG | Cloning pJT’ *Rt*PAL:Rev *Rt*PAL, *XbaI* |
| CL353 | TAACAATTTCACAGGTACCGAATTCAGGAGGTGTGACCATGTTCATTGAAACCAACG | Cloning pJT’ *Tc*XAL:Fwd *Tc*XAL, *EcoRI* |
| CL354 | GCCCGACGTCGCATGCTCCTCTAGATTAGAACATCTTGCCCAC | Cloning pJT’ *Tc*XAL:Rev *Tc*XAL, *XbaI* |
| CL349 | TAACAATTTCACAGGTACCGAATTCAGGAGGTGTGACCATGAACACCATCAACGAG | Cloning pJT’ *Fj*TAL:Fwd *Fj*TAL, *EcoRI* |
| CL350 | GCCCGACGTCGCATGCTCCTCTAGATTGTTAATCAGGTGGTCTTTC | Cloning pJT’ *Fj*TAL:Rev *Fj*TAL, *XbaI* |
| CL444 | TAACAATTTCACAGGTACCGAATTCAGGAGGTACCAGCTATGAGCCCGCCGAAGCCC | Cloning pJT’ *Rs*TAL:Fwd *Rs*TAL, *EcoRI* |
| CL445 | CACACCTCCTTCTAGATCACACCGGCGACTGCTG | Cloning pJT’ *Rs*TAL:Rev *Rs*TAL, *XbaI* |
| CL213 | TCAAGATGCTCGCTTAGAATCTAGAAGGAGGTGTGACCATGAACTACCAAAACG | Cloning pJT’ *Rt*PALa:Fwd *aroGfbr*, *XbaI* |
| CL214 | GCCCGACGTCGCATGCTCCTCTAGATTAGCCGCGCCGGGCCTT | Cloning pJT’ *Rt*PALa:Rev *aroGfbr*, *XbaI* |
| CL195 | TCAAGATGCTCGCTTAGAATCTAGAAGGAGGTGTGACCATGAAC | Cloning pJT’ *Rt*PALat:Fwd *aroGfbr-tyrAfbr*, *XbaI* |
| CL197 | GCCCGACGTCGCATGCTCCTCTAGATTACTGGCGGTTGTCGTTG | Cloning pJT’ *Rt*PALat:Fwd *aroGfbr-tyrAfbr*, *XbaI* |
| CL196 | GCCCGACGTCGCATGCTCCTCTAGATTACAGCAGTTCTTTCGC | Cloning pJT’ *Rt*PALatt:Fwd *aroGfbr-tyrAfbr-tktA*, *XbaI* |
| CL331 | TAGAAAACCTCCTTAGCATG | Cloning pBG14a/d/e/g/fg/ffg *Rt*PALatRev pBG14 backbone |
| CL332 | GAATTCGAGCTCGGTACC | Cloning pBG14a/d/e/g/fg/ffg *Rt*PALatFwd pBG14 backbone |
| CL333 | CATGCTAAGGAGGTTTTCTAATGGCACCCTCGCTCGAC | Cloning pBG14a/d/e/g/fg/ffg *Rt*PALatFwd *Rt*PAL-*aroGfbr-tyrAfbr* |
| CL334 | CGGGTACCGAGCTCGAATTCCTAAGCGAGCATCTTGAGGAGG | Cloning pBG14a/d/e/g/fg/ffg *Rt*PALatRev *Rt*PAL-*aroGfbr-tyrAfbr* |
| CL448 | TAGAAAACCTCCTTAGCATG | Cloning pBG14f *Rs*TAL at:Rev pBG14f backbone |
| CL449 | GAATTCGAGCTCGGTACC | Cloning pBG14f *Rs*TAL at:Fwd pBG14f backbone |
| CL450 | CATGCTAAGGAGGTTTTCTAATGAGCCCGCCGAAGCCC | Cloning pBG14f *Rs*TAL at:Fwd *RsTAL* |
| CL445 | CACACCTCCTTCTAGATCACACCGGCGACTGCTG | Cloning pBG14f *Rs*TAL at:Rev *RsTAL* |
| CL446 | GCCGGTGTGATCTAGAAGGAGGTGTGACCATGAAC | Cloning pBG14f *Rs*TAL at:Fwd *aroGfbr-tyrAfbr* |
| CL447 | CGGGTACCGAGCTCGAATTCTTACTGGCGGTTGTCGTTGG | Cloning pBG14f *Rs*TAL at:Rev *aroGfbr-tyrAfbr* |
| ARB6 | GGCACGCGTCGACTAGTACNNNNNNNNNNACGCC | Round 1 of arbitrary PCR |
| ARB2 | GGCACGCGTCGACTAGTAC | Round 2 of arbitrary PCR |
| pBAM-ME-I-Ext-R | CTCGTTTCACGCTGAATATGGCTC | Round 1 of arbitrary PCR for pBAMD1-2 |
| pBAM-ME-I-Ext-R | CAGTTTTATTGTTCATGATGATATA | Round 2 of arbitrary PCR for pBAMD1-2, sequencing |

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**Figure S1: Integration sites of the ferulic genes.** The operon was amplified from the genome of *P. putida* S12 and cloned into the pBELK mini-transposon vector. Tips of gene boxes indicate gene orientation, numbers indicate chromosomal location (bp). Genes are not drawn to scale. PVLB\_16205, plug domain of the TonB-dependent receptor. PVLB\_20680, SAM protein. PVLB\_25350, sensory box protein. PVLB\_16200, NAD(P)H-dependent glycerol-3-phosphate dehydrogenase. PVLB\_16210, putative ABC transporter substrate-binding protein. PVLB\_16215, integral membrane sensor hybrid histidine kinase. PVLB\_20670, XRE family transcriptional regulator. PVLB\_20675, type IV pilus biogenesis/stability protein PilW. PVLB\_20685, multifunctional nucleoside diphosphate kinase. PVLB\_25340, RipR family transcriptional regulator. PVLB\_25345, glucose-6-phosphate 1-dehydrogenase. PVLB\_23555, DNA-dependent helicase II. PVLB\_25360, hypothetical protein. *lacIq, LacI* repressor gene and Ptrc promoter*.*



**Figure S2: Investigation of 4-hydroxybenzoate production in *P. taiwanensis* VLB120 CL3 deficient for catabolite repression regulators.** Cultivations of *P. taiwanensis* VLB120 CL3 (**A**), *P. taiwanensis* VLB120 CL3 Δ*crc* (**A**) and *P. taiwanensis* VLB120 CL3 Δ*hfq* (**C**) were carried out in MSM containing 20 mM glucose as a sole carbon source in 24-well System Duetz plates at 30 °C and 300 rpm. Expression of the ferulic operon was induced with 0.2 mM IPTG. Data of biomass (circles), 4-hydroxybenzoate (squares), tyrosine (triangles) and *trans-*cinnamate (inverted triangles) are shown. Error bars indicate standard errors of the mean.

**Codon-optimized genes**

*RsTAL*

5’-ATGTTGGCCATGAGCCCGCCGAAGCCCGCTGTGGAACTGGACCGTCACATCGACCTCGATGAAGCACACTCCGTGGCCAGCGGTGGCGCTCGCATCGTCCTGGCGCCGCCAGCACGCGATCGTTGCCGCGCCAGCGAAGCCCGTCTGGGCGCCGTGATTCGTGAAGCTCGTCATGTGTACGGCCTCACCACTGGGTTCGGTCCATTGGCCAATCGGCTCGTCTCCGGTGAAAATGTCCGTACTCTGCAAGCTAACCTGGTGCATCACCTGGCGTCGGGCGTAGGCCCGGTGCTCGATTGGACCACCGCGCGGGCAATGGTCTTGGCTCGCTTGGTGGCCATCGCCCAGGGCGCGTCGGGCGCCTCGGAGGGTACGATCGCGCGCCTCATCGACCTGCTGAACAGCGAACTCGCTCCCGCCGTGCCAATGCGGGGCACCGTCGGTGCCAGCGGCGACTTGACTCCGCTGGCTCACATGGTCCTGTGCCTCCAGGGCCGCGGGGATTTCCTCGACCGGGACGGCACCCGTCTGGACGGCGCGGAGGGCCTGCGCCGCGGCCGCCTGCAACCCTTGGACCTGTCGCACCGCGACGCCCTGGCGCTGGTCAACGGCACCAGTGCCATGACCGGCATCGCGCTGGTGAACGCTCACGCTTGCCGTCACCTGGGTAATTGGGCCGTGGCTCTGACCGCGCTGCTGGCGGAGTGTCTCGGGGGCCGCACCGAAGCGTGGGCCGCCGCACTCAGCGATCTGCGCCCTCACCCCGGCCAAAAGGACGCTGCTGCTCGCCTGCGCGCGCGGGTGGACGGTAGCGCCCGCGTCGTCCGCCACGTGATTGCCGAACGCCGCCTGGGTGCGAGCGATATCGGGACTGAACCAGAAGCGGGGCAGGACGCGTACAGCCTCCGCTGCGCACCGCAGGTACTGGGCGCGGGCTTCGACACGTTGGCTTGGCATGACCGGGTCCTGACCATCGAGCTGAACGCAGTCACTGACAACCCGGTCTTCCCGCCGGACGGCAGCGTGCCGGCGCTGCACGGGGGTAACTTTATGGGCCAGCACGTCGCGCTGACCAGTGACGCACTGGCCACCGCCGTCACCGTCCTGGCCGGTCTGGCGGAGCGGCAGATCGCGCGGCTGACTGACGAACGCCTGAACCGTGGCTTGCCGCCGTTCCTGCACCGCGGCCCCGCCGGTCTCAACTCCGGCTTCATGGGCGCCCAGGTCACCGCCACCGCTCTGCTGGCAGAAATGCGCGCCACCGGCCCCGCGAGCATTCACAGCATCAGCACCAACGCCGCAAACCAGGACGTCGTCTCCCTGGGCACCATCGCAGCCCGTTTGTGCCGTGAAAAGATCGACCGCTGGGCAGAAATCCTCGCCATCCTGGCCCTGTGCCTGGCCCAGGCGGCCGAACTGCGGTGCGGCAGCGGCCTCGACGGCGTGTCGCCAGCCGGCAAGAAATTGGTCCAGGCCTTGCGCGAACAGTTCCCTCCGCTGGAGACCGATCGCCCCCTGGGGCAAGAAATCGCTGCGTTGGCCACCCACCTCTTGCAGCAGTCGCCGGTGTGA – 3’

*FjTAL*

5’-ATGAACACCATCAACGAGTATCTGTCGTTGGAGGAGTTCGAGGCCATTATTTTCGGGAACCAAAAGGTCACCATCAGCGACGTCGTCGTCAATCGCGTCAACGAATCCTTCAACTTTCTGAAGGAGTTCAGCGGCACAAGGTGATCTACGGCGTGAACACCGGCTTTGGCCCGATGGCGCAGTACCGTATCAAGGAGAGCGATCAAATCCAGCTGCAATACAACCTGATCCGCAGCCATAGCTCGGGGACCGGGAAGCCGCTGTCCCCGGTCTGTGCCAAGGCCGCTATCTTGGCCCGCCTGAACACCCTGAGCCTCGGGAACAGCGGCGTGCACCCTAGCGTAATTAATTTGATGAGCGAACTGATCAACAAGGACATCACGCCGCTGATCTTCGAACACGGCGGGGTGGGCGCGAGTGGCGACCTGGTACAGCTGTCGCACTTGGCGCTGGTCCTGATCGGCGAAGGTGAAGTCTTCTACAAAGGCGAGCGCCGTCCGACCCCGGAGGTGTTTGAAATCGAGGGCCTCAAGCCAATCCAGGTCGAGATCCGCGAAGGCCTGGCCTTGATCAACGGTACGAGCGTGATGACCGGCATCGGTGTCGTGAACGTATATCATGCGAAAAAGTTGCTGGACTGGTCGCTGAAGTCCAGCTGCGCCATCAACGAACTGGTCCAAGCCTACGACGATCACTTCAGCGCCGAACTGAACCAGACGAAACGCCACAAAGGCCAACAGGAGATCGCGCTGAAGATGCGTCAGAACCTCAGCGATTCCACCTTGATCCGGAAGCGCGAGGACCACCTGTACTCCGGCGAAAACACCGAGGAAATCTTCAAGGAAAAAGTTCAGGAGTACTACAGCCTGCGCTGTGTGCCGCAAATCCTGGGTCCTGTGCTGGAGACGATCAACAACGTTGCCTCGATCCTCGAAGATGAGTTTAACAGCGCGAATGATAACCCGATTATCGACGTCAAGAACCAACACGTCTACCACGGCGGCAACTTCCACGGCGACTACATTTCCCTGGAAATGGACAAACTGAAGATCGTGATCACCAAGCTGACGATGCTGGCCGAACGCCAACTGAATTACTTGCTGAACAGCAAGATCAATGAGCTGCTGCCCCCTTTTGTCAACCTGGGTACGCTGGGTTTCAACTTCGGTATGCAAGGGGTGCAATTTACCGCCACCAGCACCACCGCGGAATCGCAGATGCTGAGTAACCCGATGTACGTTCACTCCATTCCGAACAACAACGACAATCAGGACATTGTGAGCATGGGCACGAACTCCGCGGTAATCACCTCCAAAGTGATCGAAAACGCCTTCGAGGTACTGGCCATTGAAATGATCACCATTGTTCAGGCGATTGACTACCTGGGTCAGAAGGACAAGATCAGCTCGGTGTCGAAGAAGTGGTACGATGAAATCCGCAACATCATCCCTACGTTCAAAGAAGACCAAGTTATGTATCCTTTCGTGCAGAAGGTGAAAGACCACCTGATTAACAACTGA – 3’

*TcXAL*

5’- ATCGCGGGGGCCATCTGCGGCCACCCGGATGTCAAGGTATTCGACACCGCCGCCAGCCCACCTACCGTGCTGACCAGCCCCGAGGCAATCGCCAAATACGGCCTGAAAACCGTCAAGCTGGCCAGCAAGGAGGGCCTGGGTTTGGTGAACGGCACTGCCGTCTCGGCAGCGGCGGGCGCCTTGGCGCTGTACGATGCCGAATGCCTGGCCATCATGAGCCAGACCAACACCGTCCTGACCGTCGAAGCCCTGGACGGTCACGTAGGTAGCTTCGCGCCGTTCATCCAAGAAATCCGTCCACACGCCGGCCAAATCGAAGCTGCGCGCAACATCCGCCATATGCTCGGCGGCAGCAAATTGGCAGTCCACGAGGAATCGGAGCTGTTGGCCGACCAAGATGCAGGCATCCTGCGCCAGGACCGCTACGCTCTGCGTACCAGCGCCCAATGGATCGGGCCGCAGCTGGAGGCCCTGGGCCTGGCCCGTCAGCAGATCGAGACTGAGCTGAACTCCACCACCGATAACCCACTGATTGACGTGGAGGGGGGCATGTTCCACCATGGCGGGAACTTCCAGGCCATGGCTGTCACGTCCGCTATGGACTCCGCCCGCATCGTACTGCAGAACTTGGGCAAACTGTCCTTCGCCCAAGTAACGGAGCTGATCAACTGCGAAATGAACCATGGCCTGCCATCCAACTTGGCTGGTTCGGAACCGTCCACCAACTATCACTGCAAGGGCCTGGATATCCACTGTGGGGCGTACTGCGCCGAGCTGGGCTTCTTGGCGAACCCTATGTCGAACCATGTCCAGAGCACCGAGATGCATAACCAGAGCGTGAACTCGATGGCCTTTGCCAGCGCCCGCCGCACGATGGAAGCAAACGAAGTATTGAGCCTGCTGCTGGGCAGCCAGATGTACTGTGCCACCCAGGCCCTGGACCTGCGCGTGATGGAAGTGAAGTTCAAGATGGCAATTGTCAAACTCCTCAACGAGACGCTCACGAAGCACTTCGCCGCGTTCCTGACCCCAGAACAACTCGCCAAGCTCAATACTCATGCCGCGATCACCCTGTATAAGCGCCTGAACCAGACCCCAAGCTGGGACTCGGCGCCGCGCTTCGAAGACGCCGCCAAGCACCTGGTGGGCGTCATCATGGATGCGCTGATGGTGAACGATGATATCACCGACCTGACCAACCTCCCAAATGGAAGAAGGAGTTCGCTAAAGAGGCGGGCAACTTGTATCGCTCGATCCTCGTAGCCACCACCGCAGACGGTCGCAACGACCTGGAGCCGGCTGAGTACCTGGGCCAGACCCGCGCTGTGTACGAGGCGGTCCGGTCCGAATTGGGCGTGAAGGTCCGCCGCGGCGACGTGGCGGAGGGCAAGAGCGGTAAATCGATCGGCAGCAGCGTCGCCAAAATCGTCGAGGCGATGCGGGACGGTCGCCTGATGGGCGCGGTGGGCAAGATGTTCTGA- 3’

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5’ -

ATGAACTACCAAAACGATGATCTGCGCATCAAGGAAATCAAAGAACTCCTGCCTCCCGTAGCACTCCTGGAGAAGTTCCCAGCGACCGAGAACGCCGCCAATACCGTGGCCCACGCCCGTAAGGCCATCCACAAGATCCTCAAAGGCAACGACGATCGCTTGTTGGTCGTCATCGGGCCGTGCTCGATCCACGACCCGGTCGCGGCCAAGGAGTACGCGACCCGCCTGCTGGCGCTCCGCGAAGAGCTGAAAGACGAACTGGAAATCGTCATGCGCGTGTATTTCGAAAAGCCACGGACTACTGTGGGCTGGAAGGGTTTGATCAACGATCCTCATATGGACAACAGCTTCCAGATTAACGACGGTCTCCGCATCGCCCGCAAATTGCTGCTCGATATCAACGACAGCGGCCTGCCAGCAGCCGGCGAGTTCCTCAATATGATCACCCCCCAGTATCTCGCAGACCTGATGAGCTGGGGCGCTATTGGCGCGCGGACCACCGAGTCGCAAGTACACCGGGAACTGGCTTCGGGCCTGAGC

TGTCCTGTCGGCTTCAAGAACGGGACCGACGGCACCATCAAGGTGGCGATCGACGCCATCAACGCGGCGGGCGCCCCACACTGCTTCTTGAGTGTCACCAAGTGGGGCCACAGTGCGATTGTCAATACCTCCGGCAACGGGGACTGCCACATTATCCTGCGGGGCGGCAAGGAACCCAACTACAGCGCGAAGCACGTGGCCGAGGTTAAAGAGGGCCTCAACAAGGCTGGGCTGCCGGCCCAGGTGATGATCGACTTCAGCCATGCGAATAGCTCCAAGCAGTTCAAGAAACAGATGGATGTTTGTGCCGATGTGTGTCAGCAGATCGCCGGTGGCGAAAAAGCCATCATCGGCGTTATGGTGGAGAGCCACCTCGTCGAGGGCAACCAGTCGCTGGAAAGCGGCGAGCCTCTGGCTTACGGTAAAAGCATCACCGACGCCTGCATCGGCTGGGAGGACACCGACGCCCTGCTGCGCCAGCTGGCGAATGCCGTGAAGGCCCGGCGCGGCTAA – 3`

*tyrA*fbr

5`-

ATGGTTGCAGAACTGACCGCGCTGCGTGACCAAATCGACGAGGTGGACAAAGCACTGTTGAACCTCCTGGCTAAGCGCCTGGAGCTGGTGGCCGAGGTGGGCGAGGTCAAAAGTCGCTTTGGCCTGCCTATTTACGTTCCTGAGCGCGAGGCAAGCATCCTGGCAAGCCGTCGCGCCGAGGCTGAGGCCCTGGGTGTGCCCCCCGACTTGATCGAGGATGTCCTGCGCCGCGTGATGCGTGAAAGCTACAGCTCGGAGAACGACAAGGGCTTCAAGACCTTGTGCCCCTCCTTGCGCCCAGTCGTGATTGTCGGCGGGGGGGGCCAGATGGGCCGTTTGTTCGAAAAGATGCTCACGCTGAGCGGCTACCAGGTGCGCATCCTGGAACAGCACGACTGGGACCGGGCGGCCGACATCGTCGCTGATGCCGGTATGGTAATCGTAAGCGTCCCCATCCATGTGACCGAACAGGTGATTGGCAAGCTGCCTCCGTTGCCGAAGGACTGCATCCTGGTGGATCTCGCGAGCGTGAAGAACGGCCCGCTGCAAGCGATGCTGGTTGCCCATGATGGCCCGGTGCTGGGCCTGCACCCAATGTTCGGCCCAGACAGCGGCTCGCTGGCAAAGCAAGTCGTGGTGTGGTGCGACGGTCGCAAACCCGAGGCGTATCAATGGTTCTTGGAACAGATCCAGGTTTGGGGTGCCCGTTTGCACCGCATCTCCGCAGTCGAGCATGACCAGAATATGGCTTTCATCCAGGCCCTGCGCCATTTCGCCACCTTCGCGTACGGCCTGCACCTGGCAGAGGAGAACGTGCAGCTGGAGCAGCTGCTGGCACTCAGCTCCCCCATCTACCGCCTGGAGCTGGCCATGGTCGGTCGCCTGTTCGCCCAGGACCCGCAGCTGTACGCGGACATCATCATGTCGTCCGAACGCAATCTCGCGCTGATCAAGCGCTATTATAAACGCTTCGGCGAGGCGATCGAACTGCTGGAACAGGGCGATAAGCAAGCCTTCATCGACTCGTTCCGCAAGGTGGAACATTGGTTCGGCGACTATGTCCAGCGCTTCCAGTCGGAGTCGCGCGTTCTGTTGCGTCAAGCCAACGACAACCGCCAGTAA – 3`