

Table S2. Primers, plasmids, and strains lists.**A. Primers**

| Name | Sequence | Purpose |
|-------------------------|---|---|
| BDL3-3 | TAGAAAGCCAGTCCGCAGAAAC | PCR Linearization of pk18mobsacB |
| BDL3-4 | CTGTCGTGCCAGCTGCATTAATGAATCG | |
| BDL77-2 | TTAATGCAGCTGGCACGACAGCCTGACGAACGAGACG | PCR upstream flank for RSP1056 deletion vector |
| BDL78-2 | CCGACAGATCAGATCTTGTCCTTCCGAC | |
| BDL79-2 | GACAAGATCTGATCTGTCGGCCCCCTC | |
| BDL80-2 | CTGCGGACTGGCTTTCTACGATCCGCGCGGGCTCG | PCR downstream flank for RSP1056 deletion vector |
| BDL1-3 | ATCCCCTGATTCTGTGGATAACCGTATTACCG | PCR amplification of pk18mobsacB cloning site |
| BDL2-3 | CCCAGTCTAGCTATCGCCATGTAAGCC | |
| BDL74-1 | GCTCAACGAACTGTCGAAAC | Sequencing primers of RSP1056 deletion |
| BDL75-1 | CCGGTTCGCTTCTGATGAT | |
| BDL77-3 | TAATGCAGCTGGCACGACAGATTGAGATAGAGGAGCACG | PCR amplification of upstream flank for RSP0847 deletion vector |
| BDL78-3 | CTTAATTTGATCCGGTCACTCCTTCGTTTCGTG | |
| BDL79-3 | AGGAGTGACCGGATCAAATTAAGGATCACACTC | PCR amplification of downstream flank for RSP0847 deletion vector |
| BDL80-3 | TTCTGCGGACTGGCTTTCTAGTAGGACCACCAGGAATAGAG | |
| BDL43-3 (cenR_seq_F) | TCGATGGCGTAATGTTCTTCGC | Sequencing primers of RSP0847 deletion |
| BDL4-4 (cenR_seq_R) | GACCGAGTTGATGTTCCAGGTG | |
| BDL77-3 | TAATGCAGCTGGCACGACAGATTGAGATAGAGGAGCACG | PCR RSP0847D56A upstream flank for pk18mobsacB |
| BDL37-4 | AGACCCACGGCGAGGATCACGAGGTCGTAGAG | |
| BDL36-4 | TGATCCTCGCCGTGGGTCTGCCGGACAC | PCR RSP0847D56A downstream flank for pk18mobsacB |
| BDL11-4 | TTCTGCGGACTGGCTTTCTACCGTTGTAGCTCTTCTGTC | |
| BDL77-3 | TAATGCAGCTGGCACGACAGATTGAGATAGAGGAGCACG | PCR RSP0847D56E upstream flank for pk18mobsacB |
| BDL9-4 | AGACCCACCTCGAGGATCACGAGGTCGTAGAG | |
| BDL10-4 | TGATCCTCGAGGTGGGTCTGCCGGACAC | PCR RSP0847D56E downstream flank for pk18mobsacB |
| BDL11-4 | TTCTGCGGACTGGCTTTCTACCGTTGTAGCTCTTCTGTC | |
| BDL12-4 | CTTCCGCTTCTTCTAGCCAG | Sequencing primers for RSP0847 variants |

| | | |
|---------|---|---|
| BDL13-4 | GAAGGGCTTCGTACATAGTCG | |
| BDL1-5 | ATTTTCAGAGCGCGATCGCAGGAATGACCGCCTATGCCGAAGCG | RSP1056-cyto (truncated at M195) insertion into pVP302K with N-terminal H8-TEV tag |
| BDL2-5 | TTGTTATTTTCGGCTTTCTGTCAGACGAGGGCGCTGAGACC | |
| BDL3-5 | GAGGAGAAATTAACCATGGCTTCCCTGAAGAAGATCC | RSP0847 (WT, D56A) insertion into pVP302K with C-terminal TEV-H8 |
| BDL4-5 | TTCGCTTCCCTGCGATCGCCGCAACAAGCCTGTAGCC | |
| BDL7-5 | AAAGAGGAGAAATTAACCATGACCTCCGACACGACGC | RSP1274 insertion into pVP302K with C-terminal TEV-H8 |
| BDL8-5 | TTTTCGCTTCCCTGCGATCGCGGCGCTGAGCGTTTTCGGTCCG | |
| BDL9-5 | AAGAGGAGAAATTAACCATGGTCCCGTGCATTCTTGCGCTC | RSP1083 insertion into pVP302K with C-terminal TEV-H8 |
| BDL10-5 | TTTTCGCTTCCCTGCGATCGCGCCGGCGAAGGTGTAGCC | |
| BDL11-5 | ACATTTCCCGAAAAGTGCCAC | pVP302K sequencing primers |
| BDL12-5 | CTTTGTTAGCAGCCGGATCAGC | |
| BDL41-1 | CATATGTAATTTCTCCTC | ATW linearization of pIND5 _{spec} |
| BDL42-1 | GGATCCAGATCTCATCACCATC | |
| BDL75-3 | AAGAGGAGAAATTACATATGGCTTCCCTGAAGAAGATCC | Amplification of the coding region of <i>cenR</i> for assembly into pIND5 _{spec} |
| BDL76-3 | GATGAGATCTGGATCCTCCTCACGCAACAAGCCTGTAGCCG | |
| BDL33-1 | CTTGTGAGCGGATAACAATGATAC | Sequencing primers for pIND5 _{spec} |
| BDL34-1 | CAACCGAGCGTTCTGAACAAATCC | |
| BDL16-8 | CGAACTTCACGGTGAGCGTTG | Amplification of <i>tolQ</i> promoter region for EMSA |
| BDL17-8 | GCATCCTCCTCGATCAGGAG | |
| BDL18-8 | CAAGCTCTCGCCGAAGG | Amplification of <i>rpoH1</i> promoter region for EMSA |
| BDL19-8 | TGTAAGTGCTCATTCGTTGAC | |
| BDL20-8 | CCGTCCCTTCGACCCGCCTC | Amplification of RSP2175 promoter region for EMSA |
| BDL21-8 | GCAGGTGGCGGAACCGGGAG | |
| BDL22-8 | CAGAAAGCCGAAAATAACAAAG | Linearization of pVP302K with N-terminal H ₈ tag |
| BDL23-8 | TCCTGCGATCGCGCTCTGAAAATAC | |
| BDL24-8 | GTATTTTCAGAGCGCGATCGCAGGAATGGCTTCCCTGAAGAAGATCC | Amplification of 0847D56E gene for cloning into pVP302K-N |
| BDL25-8 | AACTTTGTTATTTTCGGCTTTCTGTCACGCAACAAGCCTGTAGC | |
| BDL26-8 | ATCCTCGAGGTGGGTCTGCCGGACACC | ATW linearization primers to make pVP302K-N-0847 from pVP302K-N-0847D56E |
| BDL27-8 | AGACCCACCTCGAGGATCACGAGGTCCG | |

B. Plasmids

| Name | Description | Source |
|-----------------------------------|---|-----------|
| pk18mobsacB | Broad host range mobilizable vector; Km ^R oriT(RP4) mobT <i>sacB lacZa</i> | [1] |
| pk18mobsacB-RSP1056 | <i>R. sphaeroides</i> genomic region flanking <i>RSP1056</i> Gibson assembled into pk18; Km ^R ; for deletion of <i>RSP1056</i> | This work |
| pk18mobsacB-RSP0847 | <i>R. sphaeroides</i> genomic region flanking <i>RSP0847</i> Gibson assembled into pk18; Km ^R ; for deletion of <i>RSP0847</i> | This work |
| pk18mobsacB-RSP0847(D56A) | <i>R. sphaeroides</i> genomic regions containing the <i>RSP0847D56A</i> allele and genomic regions flanking the gene Gibson assembled into pk18; Km ^R ; for allelic exchange within <i>RSP0847</i> | This work |
| pk18mobsacB-RSP0847(D56E) | <i>R. sphaeroides</i> genomic regions containing the <i>RSP0847D56E</i> allele and genomic regions flanking the gene Gibson assembled into pk18; Km ^R ; for allelic exchange within <i>RSP0847</i> | This work |
| pIND5 _{spec} | Replicable plasmid used for protein expression in <i>Rb. sphaeroides</i> . | [2] |
| pIND5 _{spec} -0847 | Ectopic expression of <i>RSP0847</i> (<i>cenR</i>) | This work |
| pVP302K-N/C | Protein expression vector; Km ^R lac promoter lacI, Tev site <i>rtxA</i> (<i>V. cholera</i>) coding sequence for 8×His-tag | [3] |
| pVP302K-N-RSP1056 _{cyto} | pVP302K containing truncated <i>RSP1056</i> at M195 | This work |
| pVP302K-C-RSP0847 | pVP302K containing <i>RSP0847</i> | This work |
| pVP302K-C-RSP0847(D56A) | pVP302K containing <i>RSP0847</i> variant D56A | This work |
| pVP302K-C-RSP1274 | pVP302K containing <i>RSP1274</i> | This work |
| pVP302K-C-RSP1083 | pVP302K containing <i>RSP1083</i> | This work |
| pVP302K-N-0847D56E | pVP302K containing <i>RSP0847</i> variant D56E with N-terminal H ₈ tag | This work |
| pVP302K-N-0847 | pVP302K containing <i>RSP0847</i> with N-terminal H ₈ tag | This work |

C. Strains

| Strains | Relevant characteristics | Reference |
|---|---|---------------------|
| <i>Rhodobacter sphaeroides</i> strains | | |
| 2.4.1 | Wild-type strain | ATCC 17023 |
| BDL021 | $\Delta RSP1056$ ($\Delta cenK$) | This work |
| BDL072 | <i>RSP0847D56A</i> (<i>cenR</i> D56A) | This work |
| BDL019 | <i>RSP0847D56E</i> (<i>cenR</i> D56E) | This work |
| BDL020 | <i>RSP0847D56E</i> $\Delta RSP1056$ (<i>cenR</i> D56E $\Delta cenK$) | This work |
| BDL013 | pIND5 _{spec} - <i>cenR</i> | This work |
| BDL121 | pIND5 _{spec} - <i>cenR</i> $\Delta cenR$ | This work |
| <i>Escherichia coli</i> strains | | |
| DH5 α | F- $\Phi 80lacZ\Delta M15$ $\Delta(lacZYA-argF)$ U169 <i>recA1 endA1 hsdR17</i> (rK ⁻ , mK ⁺) <i>phoA supE44</i> λ - <i>thi-1 gyrA96 relA1</i> | New England Biolabs |
| S17-1 | <i>TpR SmR recA1 thiE1 pro-82 hsdR17 RP4-2-Tc::Mu-Km::Tn7</i> λ pir | [4] |
| NEB 5-alpha competent <i>E. coli</i> | <i>fhuA2</i> $\Delta(argF-lacZ)$ U169 <i>phoA glnV44</i> $\Phi 80$ $\Delta(lacZ)$ M15 <i>gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i> | New England Biolabs |
| B834 | F- <i>hsdS metE gal ompT</i> | [5,6] |
| BL21 (DE3) pLysS CodonPlus | <i>E. coli</i> str. B F ⁻ <i>ompT gal dcm lon hsdSB</i> (rB-mB-) λ (DE3 [<i>lacI lacUV5-T7p07 ind1 sam7 nin5</i>]) [<i>malB+</i>]K-12(λ S) pLysS[T7p20 orip15A](CmR) <i>argU</i> (AGA, AGG), <i>ileY</i> (AUA), <i>leuW</i> (CUA) | Novagen |

References.

1. Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene*. 1994;145(1):69–73. doi: 10.1016/0378-1119(94)90324-7.
2. Ind AC, Porter SL, Brown MT, Byles ED, de Beyer JA, Godfrey SA, Armitage JP. Inducible Expression Plasmid for *Rhodobacter sphaeroides* and *Paracoccus denitrificans*. *Appl. Environ. Microbiol.* 2009;75:6613–6615. doi: 10.1128/AEM.01587-09.
3. Gall DL, Ralph J, Donohue TJ, Noguera DR. A Group of Sequence-Related Sphingomonad Enzymes Catalyzes Cleavage of β -Aryl Ether Linkages in Lignin β -Guaiacyl and β -Syringyl Ether Dimers. *Environ Sci Technol.* 2014;48(20):12454–63. doi: 10.1021/es503886d.
4. Simon R, Priefer U, Pühler AA. A broad range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. *Nat Biotechnol.* 1983;(1):784-791. doi: 10.1038/nbt1183-784.
5. Doherty AJ, Ashford SR, Brannigan JA, Wigley DB. A superior host strain for the over-expression of cloned genes using the T7 promoter based vectors. *Nucleic Acids Res.* 1995 Jun 11;23(11):2074-5. doi: 10.1093/nar/23.11.2074.
6. Wood WB. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. *J Mol Biol.* 1966 Mar;16(1):118-33. doi: 10.1016/s0022-2836(66)80267-x.