

Table S1. Bacterial strains and plasmids used in this study.^a

Name	Relevant characteristics ^a	Source or reference
Bacterial strain		
<i>E. coli</i> DH5 α	Cloning host; F ⁻ λ^- <i>endA1 glnX44(AS) thiE1 recA1 relA1 spoT1 gyrA96(Nal^R) rfbC1 deoR nupG</i> Φ 80(<i>lacZ</i> Δ M15) Δ (<i>argF-lac</i>)U169 <i>hsdR17(r_K⁻ m_K⁺)</i>	(1)
<i>E. coli</i> DH5 α λ <i>pir</i>	Cloning host; λ <i>pir</i> lysogen derivative of strain DH5 α	(2)
<i>E. coli</i> BL21DE3	Expression host, <i>fhuA2 [lon] ompT gal</i> (λ _{DE3}) [<i>dcm</i>] Δ <i>hsdS</i> [λ _{DE3} = λ <i>sBamHlo</i> Δ <i>EcoRI-B</i> <i>int::(lacI::P_{lacUV5::T7 gene1}) i21</i> Δ <i>nin5</i>]	New England Biolabs
<i>P. putida</i> KT2440	Wild-type strain; derivative of <i>P. putida</i> mt-2 (3) cured of the TOL plasmid pWW0	(4)
Plasmid		
pEMG	Suicide vector used for deletions in Gram-negative bacteria; <i>oriT</i> , <i>traJ</i> , <i>lacZα</i> , <i>ori</i> (R6K); Km ^R	(5)
pSEVA212s	Suicide vector used for deletions in Gram-negative bacteria; <i>oriT</i> , <i>traJ</i> , <i>lacZα</i> , <i>ori</i> (R6K); Km ^R	(6)
pSEVA628s	Helper plasmid; <i>oriV</i> (RK2), <i>XylS/Pm</i> \rightarrow <i>I-SceI</i> ; Gm ^R	(7)
pS212s $\cdot\Delta$ <i>zwfA</i>	Derivative of vector pEMG, carrying HRs to delete <i>zwfA</i> (PP_1022)	This work
pEMG $\cdot\Delta$ <i>zwfB</i>	Derivative of vector pEMG, carrying HRs to delete <i>zwfB</i> (PP_4042)	This work
pEMG $\cdot\Delta$ <i>zwf</i>	Derivative of vector pEMG, carrying HRs to delete <i>zwf</i> (PP_5351)	This work
pEMG $\cdot\Delta$ <i>hexR</i>	Derivative of vector pEMG, carrying HRs to delete <i>hexR</i> (PP_1021)	This work
pEMG $\cdot\Delta$ <i>rpiR</i>	Derivative of vector pEMG, carrying HRs to delete <i>rpiR</i> (PP_5350)	This work
pSEVA227Y	Cloning vector; <i>oriV</i> (RK2), promoter-less <i>yfp</i> ; Km ^R	(8)
pS22T \cdot P _{<i>zwfA</i>}	Derivative of vector pS227Y with 302-bp-long genomic region upstream of <i>zwfA</i>	This work
pS22T \cdot P _{<i>zwfC</i>}	Derivative of vector pS227Y with 278-bp-long genomic region upstream of <i>zwf</i>	This work
pET28a	Expression vector, <i>oriV</i> (pBR322), P _{T7} , Km ^R	Novagen
pET28a:: <i>Zwf</i>	Derivative of vector pET28a, P _{T7} \rightarrow <i>zwf</i>	This work
pET28a:: <i>ZwfB</i>	Derivative of vector pET28a, P _{T7} \rightarrow <i>zwfB</i>	This work

^a Antibiotic markers: Gm, gentamicin; Km, kanamycin; Nal, nalidixic acid; and Str, streptomycin.

REFERENCES

1. **Meselson M, Yuan R.** 1968. DNA restriction enzyme from *E. coli*. *Nature* **217**:1110-1114. <http://dx.doi.org/10.1038/2171110a0>
2. **Platt R, Drescher C, Park SK, Phillips GJ.** 2000. Genetic system for reversible integration of DNA constructs and *lacZ* gene fusions into the *Escherichia coli* chromosome. *Plasmid* **43**:12-23. <http://dx.doi.org/https://doi.org/10.1006/plas.1999.1433>
3. **Worsey MJ, Williams PA.** 1975. Metabolism of toluene and xylenes by *Pseudomonas putida* (*arvilla*) mt-2: evidence for a new function of the TOL plasmid. *J. Bacteriol.* **124**:7-13.
4. **Bagdasarian M, Lurz R, Rückert B, Franklin FCH, Bagdasarian MM, Frey J, Timmis KN.** 1981. Specific purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. *Gene* **16**:237-247. [http://dx.doi.org/10.1016/0378-1119\(81\)90080-9](http://dx.doi.org/10.1016/0378-1119(81)90080-9)
5. **Martínez-García E, de Lorenzo V.** 2011. Engineering multiple genomic deletions in Gram-negative bacteria: analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. *Environ. Microbiol.* **13**:2702-2716. <http://dx.doi.org/10.1111/j.1462-2920.2011.02538.x>
6. **Silva-Rocha R, Martínez-García E, Calles B, Chavarría M, Arce-Rodríguez A, de las Heras A, Páez-Espino AD, Durante-Rodríguez G, Kim J, Nikel PI, Platero R, de Lorenzo V.** 2013. The Standard European Vector Architecture (SEVA): a coherent platform for the analysis and deployment of complex prokaryotic phenotypes. *Nucleic Acids Res.* **41**:D666-D675. <http://dx.doi.org/10.1093/nar/gks1119>
7. **Aparicio T, Jensen SI, Nielsen AT, de Lorenzo V, Martínez-García E.** 2016. The Ssr protein (*T1E_1405*) from *Pseudomonas putida* DOT-T1E enables oligonucleotide-based recombineering in platform strain *P. putida* EM42. *Biotechnol. J.* **11**:1309-1319. <http://dx.doi.org/10.1002/biot.201600317>
8. **Martínez-García E, Aparicio T, Goñi-Moreno A, Fraile S, de Lorenzo V.** 2015. SEVA 2.0: an update of the Standard European Vector Architecture for de-/re-construction of bacterial functionalities. *Nucleic Acids Res.* **43**:D1183-D1189. <http://dx.doi.org/10.1093/nar/gku1114>