

S1 Table. Primer sequences and PCR templates used for the construction of the plasmids used in this study.

Plasmid	Primers	Sequence (5'→3') ²	PCR template ¹	Additional information ³
palk-ksa14m	PCK0017_for	ATTTAATAAAAAATTGGAGAATTCATATGG CAATTAAGAAATGCCTC	<i>ksa14m</i> gene synthesis product	insertion of <i>ksa14m</i> PCR product into pCOM10 (cut with NdeI and HindIII)
	PCK0018_rev	ATCTTCTCTCATCCGCCAAAACAGAAGCTT CGATCGTTACCCAGCCACACGTC		
palk- ksa14m-alkL	PCK0019_for	AAAGACGTGTGGGCTGGGTAACGATCGTA ACTTTAAGAAGGAGATATACCT ACTAGAT GAGTTTTTCTAATTATAAAGTAATCG	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into palk- ksa14m (cut with PvuI)
	PCK0020_rev	CATCCGCCAAAACAGAAGCTTCGATCGTTA GAAAACATATGACGCACC		
plac-ksa14m	PCK0013_for	TAGGAACCAGTACTGGAGAATTCATATG GCAATTAAGAAATGCCTC	<i>ksa14m</i> gene synthesis product	insertion of <i>ksa14m</i> PCR product into pCOM10_1A1_lac (cut with NdeI and HindIII)
	PCK0014_rev	ATCTTCTCTCATCCGCCAAAACAGAAGCTT ACTAGTTTACCCAGCCACACGTC		
plac- ksa14m-alkL	PCK0015_for	GCAAAAGACGTGTGGGCTGGGTAAC ACTAG TTAACTTTAAGAAGGAGATATACCT ACTA GATGAGTTTTTCTAATTATAAAGTAATCG	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into plac- ksa14m (cut with BcuI)
	PCK0016_rev	TTCATCCGCCAAAACAGAAGCTT ACTAGT TTAGAAAACATATGACGCACC		
ptac- ksa14m	PCK0021_for	TGACCAACAGTACTGGAGAATTCATATG GCAATTAAGAAATGCCTC	<i>ksa14m</i> gene synthesis product	insertion of <i>ksa14m</i> PCR product into pCOM10_tac (cut with NdeI and HindIII)
	PCK0018_rev	ATCTTCTCTCATCCGCCAAAACAGAAGCTT CGATCGTTACCCAGCCACACGTC		
ptac- ksa14m-alkL	PCK0019_for	AAAGACGTGTGGGCTGGGTAACGATCGTA ACTTTAAGAAGGAGATATACCT ACTAGAT GAGTTTTTCTAATTATAAAGTAATCG	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into ptac- ksa14m (cut with PvuI)
	PCK0020_rev	CATCCGCCAAAACAGAAGCTTCGATCGTTA GAAAACATATGACGCACC		
plac- ksa14m- fhuAΔ1-160	PCK0082_for	GCAAAAGACGTGTGGGCTGGGTAAC ACTAG TTAACTTTAAGAAGGAGATATACCT ACTA GATGGCGCTTCCAAAAC	pETM11_KSA14m_f huAΔ1-160 (Bertelmann <i>et al.</i> , 2022)	insertion of <i>fhuAΔ1-160</i> PCR product into plac-ksa14m-alkL (cut with BcuI)
	PCK0083_rev	TTCATCCGCCAAAACAGAAGCTT ACTAGT TTAGAAAACGGAAGGTTGC		
plac- ksa14m- todX	PCK0084_for	GCAAAAGACGTGTGGGCTGGGTAAC ACTAG TTAACTTTAAGAAGGAGATATACCT ACTA GATGAAGATTGCCAGCGTG	pETM11_KSA14m_t odX (Bertelmann <i>et</i> <i>al.</i> , 2022)	insertion of <i>todX</i> PCR product into plac-ksa14m-alkL (cut with BcuI)
	PCK0085_rev	TTCATCCGCCAAAACAGAAGCTT ACTAGT TTAAAAATTTTTGCTATAGGAAACC		
pET154	PCK0075_for	ATTGTGAGCGGATAACAATTCCTCTAGA AATAATTTGTT TAAC	<i>cyp154c5</i> gene synthesis product	insertion of <i>cyp154c5</i> PCR product into pETM11 (cut with XbaI and NotI)
	PCK0076_rev	GGTGGTGGTGGTGGTGGTCTCGAGTGC		
pET154- camAB	PCK0027_for (camA)	TGGGGCGCTAAGAGCTCCGTCGACA AAGCT TTAACTTTAAGAAGGAGATATACCAT GAA CGCAAACGACAACGTGGTC	genomic DNA of <i>Pseudomonas</i> <i>putida</i> DSM50198 (DSMZ – German Collection of Microorganisms and Cell Cultures GmbH)	simultaneous insertion of <i>camA</i> and <i>camB</i> PCR products into pET154 (cut with HindIII)
	PCK0028_rev (camA)	CTCCTTCTTAAAGTTACTCTAGTAG GATCC CTCTAGTATTAGGCACTACTCAGTTCAGCT TTG		
	PCK0029_for (camB)	CTACTAGAGTA ACTTTAAGAAGGAGATAT ACC ATGTCTAAAGTAGTGTATGTGTAC		
	PCK34_rev (camB)	GGTGTCTCGAGTGC GGCCGCAAGCTAAGCT ITTACCATTGCCTATCGGGAAC		
pET154- camAB-alkL	PCK0035_for	AGGCAATGGTAAAAGCTTAGCTT GCGGCC GCTAACTTTAAGAAGGAGATATACCAT GAA GTTTTTCTAATTATAAAGTAATCG	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into pET154- camAB (cut with NotI)
	PCK0036_rev	GGTGGTGGTGGTGGTGGTCTCGAGT GCGGC CGCTTAGAAAACATATGACGCACC		

pET154A	PCK0078_for PCK0079_rev	AGAGCTCCGTCGACAAGCTTTAAGCGGCC <u>GCTAACTTTAAGAAGGAGATATACCATGA</u> GTTTTCTAATTATAAAGTAATC GGTGGTGGTGGTGGTGGTCTCGAGTGCGGC <u>CGCTTAGAAAACATATGACGCACC</u>	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into pET154
pET106	PCK0075_for PCK0077_rev	ATTGTGAGCGGATAACAATCCCCTCTAGA AATAATTTTGT TAAAC GGTGGTGGTGGTGGTGGTCTCGAGTGCGGC <u>CGCTTAAAGCTTGTCGACGGAG</u>	<i>cyp106a2</i> gene synthesis product	insertion of <i>cyp106a2</i> PCR product into pETM11
pET106A	PCK0078_for PCK0079_rev	AGAGCTCCGTCGACAAGCTTTAAGCGGCC <u>GCTAACTTTAAGAAGGAGATATACCATGA</u> GTTTTCTAATTATAAAGTAATC GGTGGTGGTGGTGGTGGTCTCGAGTGCGGC <u>CGCTTAGAAAACATATGACGCACC</u>	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product into pET106
ptac154	PCK0063_for PCK0064_rev	AGTCCGTTTAGGTGTTTTACGAGCAATTG CCTCTAGAAATAATTTTGT TAAAC TGGCTGCAGGTGCACGGATCCC GGGCGC <u>GCCTTAAAGCTTGTCGACGG</u>	<i>cyp154c5</i> gene synthesis product	insertion of <i>cyp154c5</i> PCR product in pCOM10_tac (cut with MnlI and SgsI)
ptac154A	PCK0065_for PCK0066_rev	AGAGCTCCGTCGACAAGCTTTAAGCGCGC <u>CCTAACTTTAAGAAGGAGATATACCATGA</u> GTTTTCTAATTATAAAGTAATC ACAGAAGCTTGGCTGCAGGTGCACGGATC <u>CTTAGAAAACATATGACGCACC</u>	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product in ptac154 (cut with SgsI and BamHI)
ptac106	PCK0067_for PCK0068_rev	AGTCCGTTTAGGTGTTTTACGAGCAATTG CCTCTAGAAATAATTTTGT TAACTTTAAG AAGGAGATATACCATGA AGGAGGTGATCG CAG TGGCTGCAGGTGCACGGATCCC GGGCGC <u>GCCTTAAAGCTTGTCGACGGAGCTTTACA</u> TACGACTCGCTTTCAG	<i>cyp106a2</i> gene synthesis product	insertion of <i>cyp106a2</i> PCR product in pCOM10_tac (cut with MnlI and SgsI)
ptac106A	PCK0065_for PCK0066_rev	AGAGCTCCGTCGACAAGCTTTAAGCGCGC <u>CCTAACTTTAAGAAGGAGATATACCATGA</u> GTTTTCTAATTATAAAGTAATC ACAGAAGCTTGGCTGCAGGTGCACGGATC <u>CTTAGAAAACATATGACGCACC</u>	pCW3A4LS (derived from pLAFR3-AlkL (Cornelissen <i>et al.</i> , 2013))	insertion of <i>alkL</i> PCR product in ptac106 (cut with SgsI and BamHI)
pACYC- camA	PCK0059_for PCK0060_rev	GTTAACTTTAAT AAGGAG ATATACCATG AACGCAAACGACAACGTGGTC TGTCGACCTGCAGGCGCGCCGAGCTCTCA GGCACTACTCAGTTCAGCTTTG	genomic DNA of <i>Pseudomonas</i> <i>putida</i> DSM50198 (DSMZ – German Collection of Microorganisms and Cell Cultures GmbH)	insertion of <i>camA</i> PCR product into pACYC_DuetI (cut with NcoI and SacI)
pACYC- camAB	PCK0061_for PCK0062_rev	GTTAAGTATAAG AAGGAG ATATACATATGT CTAAAGTAGTGTATGTGTACAC GCGATCGCGTGGCCGCGCATATCCAATT <u>GTTACCATTGCCTATCGGGAAAC</u>	genomic DNA of <i>Pseudomonas</i> <i>putida</i> DSM50198 (DSMZ – German Collection of Microorganisms and Cell Cultures GmbH)	insertion of <i>camB</i> PCR product into pACYC-camA (cut with NdeI and MnlI)

¹ Genes encoding the respective CYP450s, redox partner proteins, or outer membrane proteins were amplified from various sources using suitable primers.

² Primers contained (parts of) ribosomal binding sites (**bold**), restriction sites, and 25 bp overhangs complementary to the plasmid backbone.

³ The resulting PCR products were inserted into the different vectors by *in vitro assembly* (Gibson *et al.*, 2009). Final plasmid constructs were then introduced into the desired host organism.

References

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