Supplementary material

Functional genetic elements for controlling gene expression in *Cupriavidus necator* H16

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The authors wish it to be known that the first three authors, Swathi Alagesan, Erik K. R. Hanko and Naglis Malys, should be regarded as joint First Authors

SUPPLEMENTARY MATERIAL AND METHODS

Construction of modular vector pMTL71101

Vector pMTL71101 was built on the basis of plasmid pMTL83141, which is formed of four modules flanked by 8 bp recognition sites of the type II restriction endonucleases AscI, FseI, PmeI and SbfI (1). Three modifications using plasmid pMTL83141 were carried out as following. First, the 892 bp PmeI-SbfI-flanked DNA fragment encoding the *E. coli* Gram negative replicon ColEI RNA II was replaced with a fragment containing broad-host-range origin of replication (pBBR1) and mobilisation gene *mob*. This 4045 bp DNA fragment was amplified by PCR using primers P015_71101_f and P016_71101_r from plasmid pME6000 (2), followed by restriction digestion with PmeI and SbfI. Second, the 2418 bp AscI-PmeI-flanked DNA fragment including the Gram positive replicon pCB102 and chloramphenicol resistance marker (*catP*) was replaced with a 805 bp DNA fragment containing solely *catP*, which was amplified by PCR using primers P017_CatP_f and P018_CatP_r from pMTL83141, followed by restriction digestion with AscI and PmeI. Third, the NotI restriction site in the *mob* gene locus was removed by conservative change using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies) and primers P019_Qmob_f and P020_Qmob_r by following manufacturer's instructions. The resulting plasmid pMTL71101 contains the pBBR1 origin of replication and mobilisation gene *mob* (PmeI-SbfI module), the chloramphenicol resistance marker *catP* (FseI-PmeI module) and a multiple cloning site (SbfI-AscI module) (Supplementary Figure S1).

Codon-optimised *P. alba ispS* gene encoding for isoprene synthase, 49 amino acids truncated at N-terminus

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ATGGAAGCCCGCCGCTCGGCCAACTACGAGCCGAACTCGTGGGACTACGACTACCTGCTGTC
GTCGGACACCGACGAGTCGATCGAGGTGTACAAGGACAAGGCCAAGAAGCTGGAAGCCGAGG
TCCGCCGCGAGATCAACAACGAGAAGGCCGAGTTCCTGACGCTGCTGGAACTGATCGACAAC
GTGCAGCGCCTGGGCCTGGGCTACCGCTTCGAAAGCGACATCCGCGGCGCCCTGGACCGCTT
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CGTGAGCAGCGGCGGCTTCGACGCCGTGACCAAGACCTCGCTGCATGGCACCGCGCTGTCGT
TCCGCCTGCTGCGCCAGCACGGCTTCGAGGTGTCGCAGGAAGCCTTCTCGGGCTTCAAGGAC
CAGAACGGCAACTTCCTGGAAAACCTGAAGGAAGATATCAAGGCCATCCTGTCGCTGTACGA
GGCCAGCTTCCTGGCGCTGGAAGGCGAGAACATCCTGGACGAGGCCAAGGTGTTCGCCATCT
CGCATCTGAAGGAACTGTCGGAGGAAAAGATCGGCAAGGAACTGGCCGAACAGGTGAACCAT
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CTACCGCAAGAAGGAAGACGCCAACCAGGTCCTGCTGGAACTGGCCATCCTGGACTACAACA
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CGTCGCCTTCGAGCCGCAGTATTCGGACTGCCGCAACTCGGTGGCCAAGATGTTCAGCTTCG
TGACCATCATCGACGACATCTACGACGTGTACGGCACCCTGGACGAACTGGAACTGTTCACC
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ACCTGATCGACGAAACCTGGAAGAAGATGAACAAGGAAAAGCTGGGCGGCTCGCTGTTCGCC
AAGCCGTTCGTGGAAACCGCGATCAATCTGGCCCGCCAGTCGCACTGCACCTACCACAACGG
CGACGCGCACACCTCGCCGGATGAGCTGACCCGCAAGCGCGTGCTGAGCGTGATCACCGAGC
CGATCCTGCCGTTCGAGCGCTGA

SUPPLEMENTARY TABLES

Supplementary Table S1. Oligonucleotide primers used in this study.

Primer	Sequence 5' to 3'
P001	atatggtaccaaattaagcagaaggccatcctgacggatggccttttgcgtttcttaaggctgagggaaagtctagacgattcattaatgcagctggc
P002	aattaccggtaaacaacagataaaacgaaaggcccgaaggcctttcgttttatttgatgctagctgaggagacatctagaaaggaagggaagaaagcg
P003	gggaaaguacccaaaaattcatccttctcg
P004	ggagacauggatccaagcttacttgtacagctcgtccatgc
P005_p0_1_r	attgtctcuctgccgtcactattcg
P005_p0_1-sd_r	attgagagugagccgtcactattcgaaccg
P005_p0_1+SD_r	agtgaattcugacatttgagtccattgttgcc
P005_p0_2_r	attgtctcuctgccgacatttgagtccattgttgcc
P005_p1_r	attataattgutatccgctcacaaagcaaataaatttttttatcgattaatccgcctcggcactgc
P005_p2_r	aaagtcaguctagggtagtcactatcatcgtgcaagttcgtttttaatccgcctcggcactgc
P005_p3_r	attcattauacgagccgatgattaattgtcaacagctcatcgattaatccgcctcggcactgc
P005_p4_r	agtatattcuaaatttccacctgtgtcaataacggttttatcgattaatccgcctcggcactgc
P005_p5_r	acgagaauttgaagcgtttagcaaatgaatttttttaatcgattaatccgcctcggcactgc
P005_p6_r	attctattauacagaaaaattttcctgaaagcaaataaatttttttatcgattaatccgcctcggcactgc
P005_p7_r	aggtctataauaccacaatagtatcagtatgtaaacaactttctgaaatttttttatcgattaatccgcctcggcactgc
P005_p8_r	atgaatctautatatcgccgcaagggataaaaagcaaataaatttttttatcgattaatccgcctcggcactgc
P005_p9_r	atgaatctautataggtacaaaaagatgcgaagtcaatactctttttatcgattaatccgcctcggcactgc
P005_p10_r	atcttcaauattgagtggcacggttaagctccaaaatgacgatcgattaatccgcctcggcactgc
P005_p11_r	acgttaaauctaggtacaaaaagatgcgaaagcaaataaatttttttatcgattaatccgcctcggcactgc
P005_p12_r	agcacaauacctaggactgagctagccgtcaaatcgattaatccgcctcggcactgc
P005_p13_r	acacattauacgagccggatgattaaaagggaaatcgattaatccgcctcggcactgc
P005_p14_r	attcgtcccuccacacatcctaggagccggatgattaattgtcaaatcgattaatccgcctcggcactgc
P005_p15_r	attcaggtugctagcacagtacctaggactgagctagctgtcaaatcgattaatccgcctcggcactgc
P005_p16_r	attaagcuagcactgtacctaggactgagctagccgtcaaatcgattaatccgcctcggcactgc

P005_p17_r	atgaaataautatgggtacaaaaagatgcgatgtcaaatcgattaatccgcctcggcactgc
P005_p18_r	atgaaataautatgcctataggttagactttatgtcaaatcgattaatccgcctcggcactgc
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P006_p0_1_f	agagacaaucaacatatggtgagc
P006_p0_1-sd_f	actctcaaucaacatatggtgagcaagg
P006_p0_1+SD_f	agaattcacuagtttaactttaagaaggagatatacatatggtgagcaagggc
P006_p0_2_f	agagacaaucaacatatggtgagc
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P008_rbs14_f	aaggaggtuaacatatggtgagcaagggcgag
P008_rbs20_f	agagagacaaucaacatatggtgagcaagggcgag
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P008_rbs24_f	aggaggaaguatggtgagcaagggcgag
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P008_rbs27_f	aaggagauttagatggtgagcaagggcgag
P008_rbs28_f	aggagatauagatggtgagcaagggcgag
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EH109_r	tttaagaaggagatatacatatggcgagtagc
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EH112_f	ccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaaacagctatggtgatcatgagtccaaagagaa
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EH114_f	atgtatatctccttcttaaagttaaacaaaattatttctagtaac
EH310_f	gctactcgccatatgtatatctccttcttaaaagatcttttgaattcccaaaaaaacgggtatggagaaac

Supplementary Table S2. Plasmids used and generated in this study.

Plasmid	Characteristic	Reference or source
pMTL83141	Cm^r, ColE1 RNA II, CatP, *lacZ, pCB102*	(1)
pME6000	Tet^r, pBBR1 ori, *lacZ, mob*$^+$	(2)
pBBR1MCS-2-P*phaC-eyfp-c1*	Kan^r; broad host range vector used as a backbone and to amplify the eyfp gene	(3)
pGEM-T	Amp^r; *E. coli* vector used to amplify the *lacZ* gene for blue/white colony screening	Promega
pEH006	Cm^r; P_{araC}-*araC*-T_{rrnB1} and P_{araBAD}-T7sl-*Ec*RBS-*rfp*-T_{dbl}	(4)
pJOE7784.1	Kan^r; vector used to amplify P_{rhaSR}-*rhaSR* and P_{rhaBAD}	(5)
pJOE7801.1	Kan^r; vector used to amplify *tetR*	(5)
pMTL71101	Cm^r; broad host range modular vector used as a backbone	This study
pBBR1MCS-2-USER	Kan^r; broad host range vector with USER cassette	This study
pMTL71107	Cm^r; broad host range modular vector with USER cassette	This study
pBBR1MCS-2-P_0	Kan^r; P_0-*eyfp*, assembled from pBBR1MCS-2-USER1 and PCR fragments generated by PCR using primer pair P006_P0_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pBBR1MCS-2-P_{0_1}	Kan^r; P_{0_1}-*eyfp*, assembled from pBBR1MCS-2-USER1 and PCR fragments generated by PCR using primer pairs P003-P005_P0_1_r and P006_P0_1_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pBBR1MCS-2-$P_{0\_1\text{-}sd}$	Kan^r; $P_{0\_1\text{-}sd}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{0_1+SD}	Kan^r; P_{0_1+SD}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-P_{0_2}	Kan^r; P_{0_2}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_1	Kan^r; P_1-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_2	Kan^r; P_2-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_3	Kan^r; P_3-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_4	Kan^r; P_4-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_5	Kan^r; P_5-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_6	Kan^r; P_6-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_7	Kan^r; P_7-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_8	Kan^r; P_8-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_9	Kan^r; P_9-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{10}	Kan^r; P_{10}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{11}	Kan^r; P_{11}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-P_{12}	Kan^r; P_{12}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{13}	Kan^r; P_{13}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{14}	Kan^r; P_{14}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{15}	Kan^r; P_{15}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{16}	Kan^r; P_{16}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{17}	Kan^r; P_{17}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{18}	Kan^r; P_{18}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{19}	Kan^r; P_{19}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{20}	Kan^r; P_{20}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{21}	Kan^r; P_{21}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{22}	Kan^r; P_{22}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{23}	Kan^r; P_{23}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-P_{24}	Kan^r; P_{24}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-P_{25}	Kan^r; P_{25}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_0	Cm^r; P_0-*eyfp*, assembled from pMTL71107 and PCR fragments generated by PCR using primer pair P006_P0_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pMTL71101-P_{0_1}	Cm^r; P_{0_1}-*eyfp*, assembled from pMTL71107 and PCR fragments generated by PCR using primer pairs P003-P005_P0_1_r and P006_P0_1_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pMTL71101-$P_{0\_1\text{-}sd}$	Cm^r; $P_{0\_1\text{-}sd}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{0_1+SD}	Cm^r; P_{0_1+SD}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{0_2}	Cm^r; P_{0_2}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_1	Cm^r; P_1-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_2	Cm^r; P_2-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_3	Cm^r; P_3-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_4	Cm^r; P_4-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_5	Cm^r; P_5-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pMTL71101-P_6	Cm^r; P_6-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_7	Cm^r; P_7-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_8	Cm^r; P_8-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_9	Cm^r; P_9-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{10}	Cm^r; P_{10}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{11}	Cm^r; P_{11}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{12}	Cm^r; P_{12}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{13}	Cm^r; P_{13}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{14}	Cm^r; P_{14}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{15}	Cm^r; P_{15}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{16}	Cm^r; P_{16}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{17}	Cm^r; P_{17}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pMTL71101-P_{18}	Cm^r; P_{18}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{19}	Cm^r; P_{19}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{20}	Cm^r; P_{20}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{21}	Cm^r; P_{21}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{22}	Cm^r; P_{22}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{23}	Cm^r; P_{23}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{24}	Cm^r; P_{24}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-P_{25}	Cm^r; P_{25}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS_0	Kan^r; P_{phaC}-RBS_0-*eyfp*, assembled from pBBR1MCS-2-USER1 and PCR fragments generated by PCR using primer pairs P003-P007_rbs0_r and P008_rbs0_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pBBR1MCS-2-RBS_1	Kan^r; P_{phaC}-RBS_1-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS_2	Kan^r; P_{phaC}-RBS_2-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-RBS$_3$	Kan^r; P$_{phaC}$-RBS$_3$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_4$	Kan^r; P$_{phaC}$-RBS$_4$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_5$	Kan^r; P$_{phaC}$-RBS$_5$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_6$	Kan^r; P$_{phaC}$-RBS$_6$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_7$	Kan^r; P$_{phaC}$-RBS$_7$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_8$	Kan^r; P$_{phaC}$-RBS$_8$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_9$	Kan^r; P$_{phaC}$-RBS$_9$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{10}$	Kan^r; P$_{phaC}$-RBS$_{10}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{11}$	Kan^r; P$_{phaC}$-RBS$_{11}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{12}$	Kan^r; P$_{phaC}$-RBS1$_2$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{13}$	Kan^r; P$_{phaC}$-RBS$_{13}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{14}$	Kan^r; P$_{phaC}$-RBS$_{14}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-RBS$_{20}$	Kan^r; P$_{phaC}$-RBS$_{20}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{21}$	Kan^r; P$_{phaC}$-RBS$_{21}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{22}$	Kan^r; P$_{phaC}$-RBS$_{22}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{23}$	Kan^r; P$_{phaC}$-RBS$_{23}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{24}$	Kan^r; P$_{phaC}$-RBS$_{24}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{25}$	Kan^r; P$_{phaC}$-RBS$_{25}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{26}$	Kan^r; P$_{phaC}$-RBS$_{26}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{27}$	Kan^r; P$_{phaC}$-RBS$_{27}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{28}$	Kan^r; P$_{phaC}$-RBS$_{28}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{29}$	Kan^r; P$_{phaC}$-RBS$_{29}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{30}$	Kan^r; P$_{phaC}$-RBS$_{30}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{31}$	Kan^r; P$_{phaC}$-RBS$_{31}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pBBR1MCS-2-RBS$_{32}$	Kan^r; P$_{phaC}$-RBS$_{32}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-SL/RBS$_1$	Kan^r; P$_{phaC}$-T7*g10* mRNA stemloop-RBS$_1$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-SL/RBS$_2$	Kan^r; P$_{phaC}$-T7*g10* mRNA stemloop-RBS$_2$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-SL/RBS$_8$	Kan^r; P$_{phaC}$-T7*g10* mRNA stemloop-RBS$_8$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_4$/RBS$_1$	Kan^r; P$_{phaC}$-(A/U)$_4$-RBS$_1$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_6$/RBS$_1$	Kan^r; P$_{phaC}$-(A/U)$_6$-RBS$_1$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_9$/RBS$_1$	Kan^r; P$_{phaC}$-(A/U)$_9$-RBS$_1$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_{12}$/RBS$_1$	Kan^r; P$_{phaC}$-(A/U)$_{12}$-RBS$_1$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_{12}$/RBS$_2$	Kan^r; P$_{phaC}$-(A/U)$_{12}$-RBS$_2$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-(A/U)$_{12}$/RBS$_8$	Kan^r; P$_{phaC}$-(A/U)$_{12}$-RBS$_8$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_1$-ispS	Kan^r; P$_{phaC}$-RBS$_1$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_1$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_2$-ispS	Kan^r; P$_{phaC}$-RBS$_2$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_2$ as described in *Material and Methods*	This study

pBBR1MCS-2-RBS$_{5}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{5}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{5}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{6}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{6}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{6}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{8}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{8}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{8}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{9}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{9}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{9}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{13}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{13}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{13}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{14}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{14}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{14}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{20}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{20}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{20}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{24}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{24}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{24}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{25}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{25}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{25}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{28}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{28}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{28}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{31}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{31}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{31}$ as described in *Material and Methods*	This study
pBBR1MCS-2-RBS$_{32}$-ispS	Kan^{r}; P$_{phaC}$-RBS$_{32}$-*ispS*, constructed by replacing *eyfp* with *ispS* in pBBR1MCS-2-RBS$_{32}$ as described in *Material and Methods*	This study

pBBR1MCS-2-P_{1}-RBS_{1}	Kan^{r}; P_{1}-RBS_{1}-*eyfp,* constructed by replacing P_{phaC} with P_{1} in pBBR1MCS-2-RBS_{1}	This study
pBBR1MCS-2-P_{1}-RBS_{2}	Kan^{r}; P_{1}-RBS_{2}-*eyfp,* constructed by replacing P_{phaC} with P_{1} in pBBR1MCS-2-RBS_{2}	This study
pBBR1MCS-2-P_{1}-RBS_{8}	Kan^{r}; P_{1}-RBS_{8}-*eyfp,* constructed by replacing P_{phaC} with P_{1} in pBBR1MCS-2-RBS_{8}	This study
pBBR1MCS-2-P_{rhaBAD}-RBS_{1}	Kan^{r}; P_{rhaBAD}-RBS_{1}-*eyfp,* constructed by replacing P_{phaC} with P_{rhaBAD} in pBBR1MCS-2-RBS_{1}	This study
pBBR1MCS-2-P_{rhaBAD}-RBS_{2}	Kan^{r}; P_{rhaBAD}-RBS_{2}-*eyfp,* constructed by replacing P_{phaC} with P_{rhaBAD} in pBBR1MCS-2-RBS_{2}	This study
pBBR1MCS-2-P_{rhaBAD}-RBS_{8}	Kan^{r}; P_{rhaBAD}-RBS_{8}-*eyfp,* constructed by replacing P_{phaC} with P_{rhaBAD} in pBBR1MCS-2-RBS_{8}	This study
pBBR1MCS-2-RBS_{1}-rfp	Kan^{r}; P_{phaC}-RBS_{1}-*rfp,* constructed by replacing *eyfp* with *rfp* in pBBR1MCS-2-RBS_{1}	This study
pBBR1MCS-2-RBS_{2}-rfp	Kan^{r}; P_{phaC}-RBS_{2}-*rfp*, constructed by replacing *eyfp* with *rfp* in pBBR1MCS-2-RBS_{2}	This study
pBBR1MCS-2-RBS_{8}-rfp	Kan^{r}; P_{phaC}-RBS_{8}-*rfp*, *constructed by replacing eyfp with rfp* in pBBR1MCS-2-RBS_{8}	This study
pMTL71101-RBS_{0}	Cm^{r}; P_{phaC}-RBS_{0}-*eyfp*, assembled from pMTL71107 and PCR fragments generated by PCR using primer pairs P003-P007_rbs0_r and P008_rbs0_f-P004 from pBBR1MCS-2-PphaC-eyfp-c1	This study
pMTL71101-RBS_{1}	Cm^{r}; P_{phaC}-RBS_{1}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS_{2}	Cm^{r}; P_{phaC}-RBS_{2}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS_{3}	Cm^{r}; P_{phaC}-RBS_{3}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS_{4}	Cm^{r}; P_{phaC}-RBS_{4}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS_{5}	Cm^{r}; P_{phaC}-RBS_{5}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS_{6}	Cm^{r}; P_{phaC}-RBS_{6}-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

pMTL71101-RBS$_{7}$	Cm^{r}; P$_{phaC}$-RBS$_{7}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{8}$	Cm^{r}; P$_{phaC}$-RBS$_{8}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{9}$	Cm^{r}; P$_{phaC}$-RBS$_{9}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{10}$	Cm^{r}; P$_{phaC}$-RBS$_{10}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{11}$	Cm^{r}; P$_{phaC}$-RBS$_{11}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{12}$	Cm^{r}; P$_{phaC}$-RBS$_{12}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{13}$	Cm; P$_{phaC}$-RBS$_{13}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{14}$	Cm^{r}; P$_{phaC}$-RBS$_{14}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{20}$	Cm^{r}; P$_{phaC}$-RBS$_{20}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{21}$	Cm^{r}; P$_{phaC}$-RBS$_{21}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{22}$	Cm^{r}; P$_{phaC}$-RBS$_{22}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{23}$	Cm^{r}; P$_{phaC}$-RBS$_{23}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study

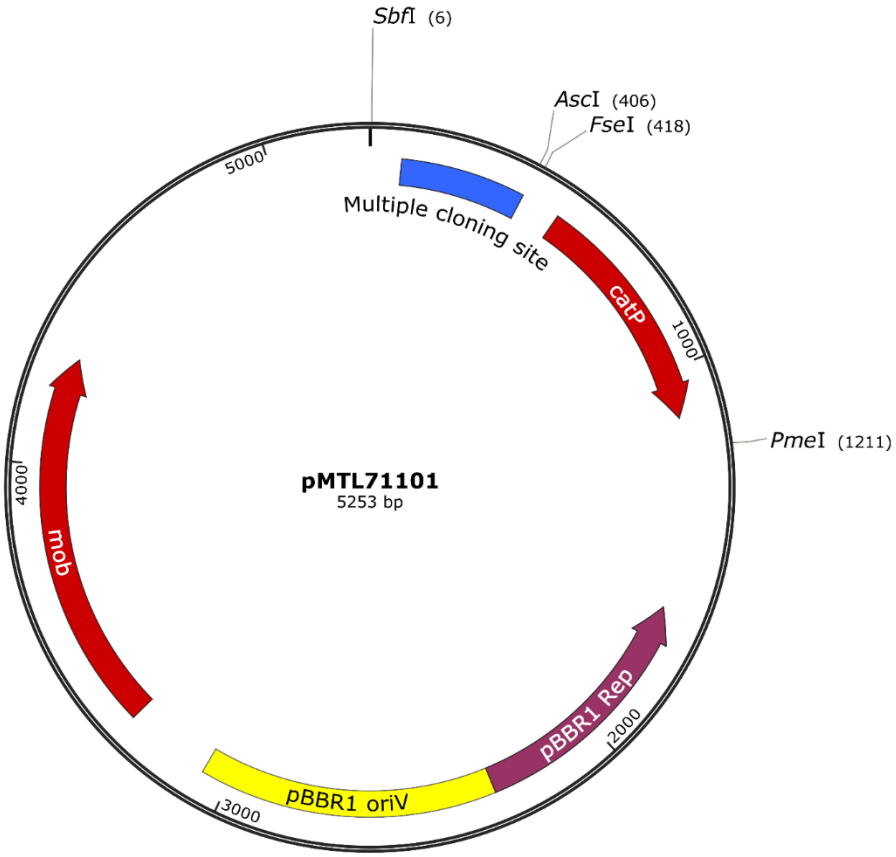
pMTL71101-RBS$_{24}$	Cm^r; P$_{phaC}$-RBS$_{24}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{25}$	Cm^r; P$_{phaC}$-RBS$_{25}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{26}$	Cm^r; P$_{phaC}$-RBS$_{26}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{27}$	Cm^r; P$_{phaC}$-RBS$_{27}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{28}$	Cm^r; P$_{phaC}$-RBS$_{28}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{29}$	Cm; P$_{phaC}$-RBS$_{29}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{30}$	Cm^r; P$_{phaC}$-RBS$_{30}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{31}$	Cm^r; P$_{phaC}$-RBS$_{31}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pMTL71101-RBS$_{32}$	Cm^r; P$_{phaC}$-RBS$_{32}$-*eyfp*, assembled as above using corresponding primers as described in *Material and Methods*	This study
pEH002	Cm^r; P$_{rhaSR}$-*rhaSR*-T$_{rrnB1}$ and P$_{rhaBAD}$-T7sl-*Ec*RBS-*rfp*-T$_{dbl}$ from pJOE7784.1 cloned into pEH006 by AatII and XbaI sites	This study
pEH005	Cm^r; P$_{lac}$-*tetR*-T$_{rrnB2}$ and P$_{tetA}$-T7sl-*Ec*RBS-*rfp*-T$_{dbl}$ from pJOE7801.1 assembled by NEBuilder	This study
pEH020	Cm^r; P$_{lac}$-*acuR*-T$_{rrnB2}$ and P$_{acuR}$-T7sl-*Ec*RBS-*rfp*-T$_{dbl}$ from *R. sphaeroides* 2.4.1 genomic DNA assembled by NEBuilder	This study

pEH040	Cm^r; P$_{lac}$-*cymR*-T$_{rrnB2}$ and P$_{cmt}$-*Ec*RBS-*rfp*-T$_{dbl}$ from pNEW assembled by NEBuilder	This study
pEH002-ispS	Cm^r; P$_{rhaSR}$-*rhaSR*-T$_{rrnB1}$ and P$_{rhaBAD}$-T7sl-*Ec*RBS-*ispS*-T$_{dbl}$, *rfp* replaced with *ispS* in pEH002 through NdeI and BamHI restriction sites	This study
pEH005-ispS	Cm^r; P$_{lac}$-*tetR*-T$_{rrnB2}$ and P$_{tetA}$-T7sl-*Ec*RBS-*rfp*-T$_{dbl}$, *rfp* replaced with *ispS* in pEH002 through NdeI and BamHI restriction sites	This study
pEH020-ispS	Cm^r; P$_{lac}$-*acuR*-T$_{rrnB2}$ and P$_{acuR}$-T7sl-*Ec*RBS-*rfp*-T$_{dbl}$, *rfp* replaced with *ispS* in pEH002 through NdeI and BamHI restriction sites	This study
pEH040-ispS	Cm^r; P$_{lac}$-*cymR*-T$_{rrnB2}$ and P$_{cmt}$-*Ec*RBS-*rfp*-T$_{dbl}$, *rfp* replaced with *ispS* in pEH002 through NdeI and AflII restriction sites	This study
pEH176	Cm^r; P*araC*-*araC*-T$_{rrnB1}$ and P*araBAD*-*Ec*RBS-*rfp*-T$_{dbl}$ from *E. coli* MG1655 genomic DNA cloned into pEH006 by AatII and NdeI sites	This study

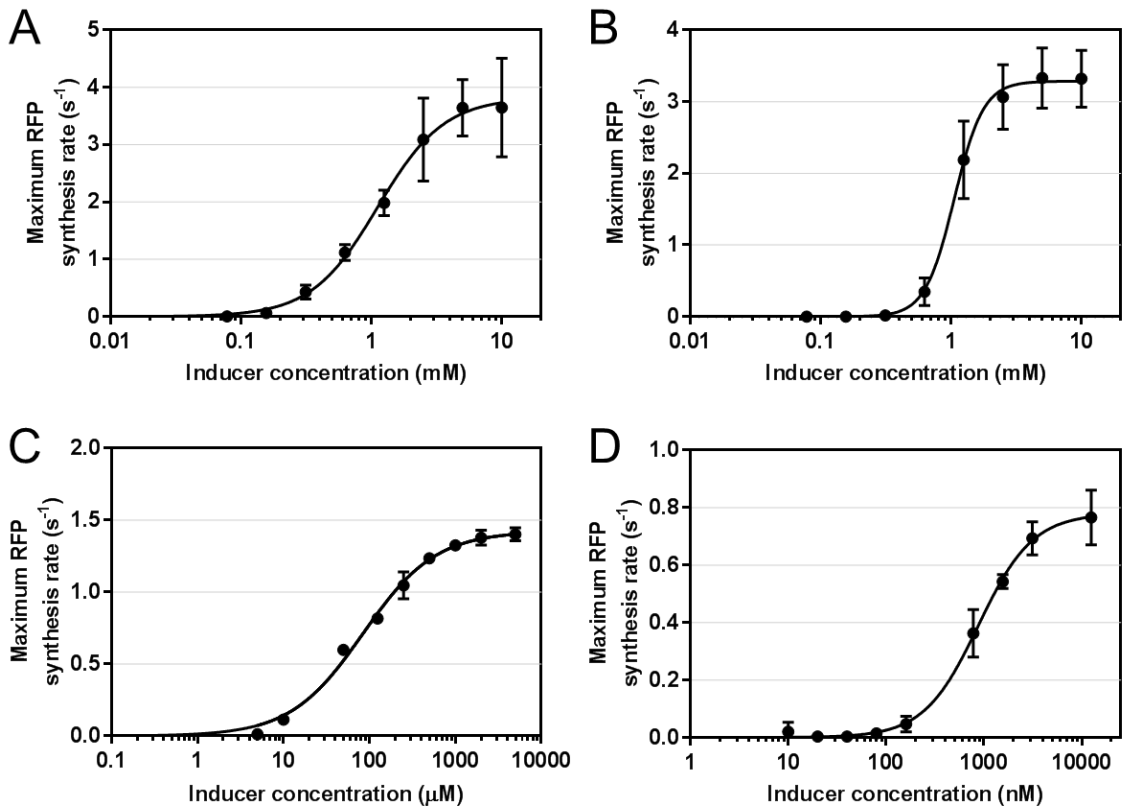
Supplementary Table S3. Hill function fitting parameters.

Plasmid identifier	Inducer	Minimum RFP synthesis rate, v_{min} (s^{-1})	Maximum RFP synthesis rate, v_{max} (s^{-1})	Hill coefficient, h	Half-maximal RFP synthesis, K_m (µM)
pEH002	L-rhamnose	0.0058 ± 0.0022	3.292 ± 0.032	3.88 ± 0.23	1057 ± 19
pEH006	L-arabinose	0.0122 ± 0.0023	3.836 ± 0.085	1.70 ± 0.11	1116 ± 56
pEH020	acrylate	0.0474 ± 0.0056	1.471 ± 0.035	1.03 ± 0.09	84.32 ± 8.08
pEH040	cumate	0.0472 ± 0.0076	0.828 ± 0.011	1.56 ± 0.08	0.877 ± 0.029

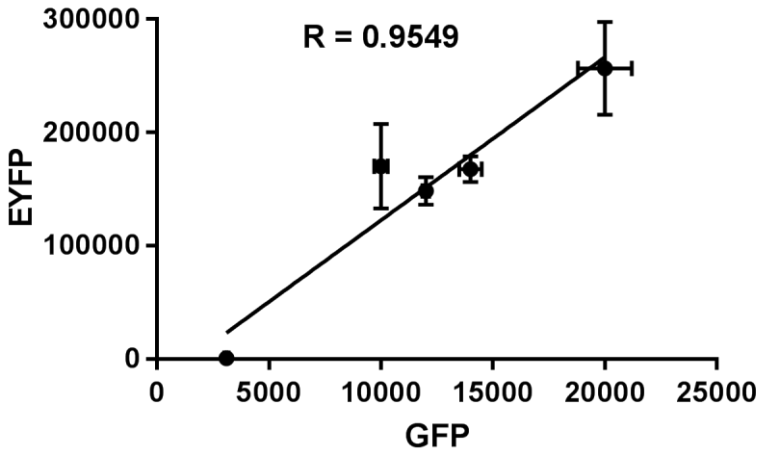
SUPPLEMENTARY FIGURES



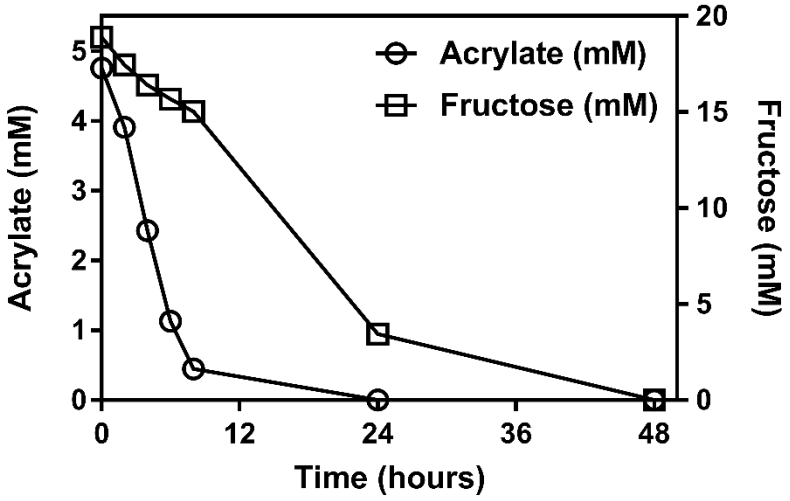
Supplementary Figure S1. Plasmid map of modular vector pMTL71101. Restriction endonucleases SbfI, AscI, FseI, and PmeI, flanking modules of the vector, are shown with their restriction site positions in the brackets. The pBBR1 origin of replication and mobilisation gene *mob* (PmeI-SbfI module), the chloramphenicol resistance marker *catP* (FseI-PmeI module) and a multiple cloning site (SbfI-AscI module) are highlighted.



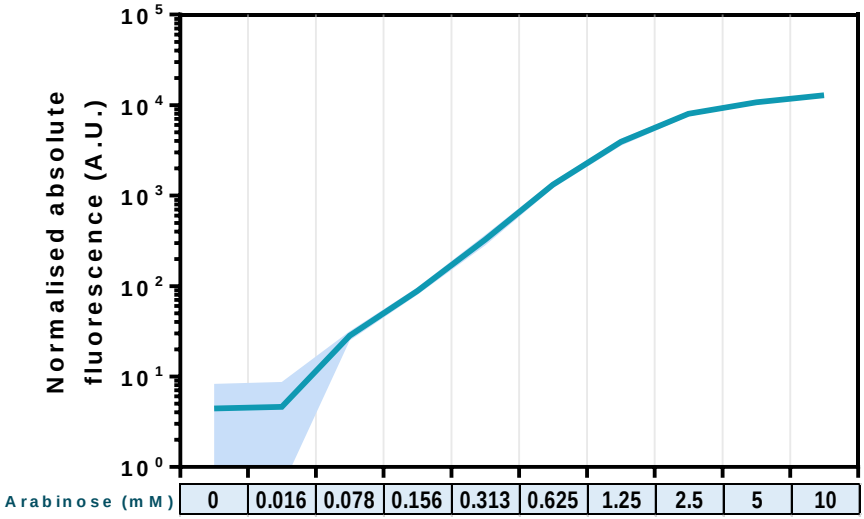
Supplementary Figure S2. Promotor activity of inducible systems. The normalised maximum rate of RFP synthesis was fit to the corresponding inducer concentration using a Hill function for the (A) L-arabinose-, (B) L-rhamnose-, (C) acrylate-, and (D) cumate-inducible systems. Error bars represent standard deviations of three biological replicates.



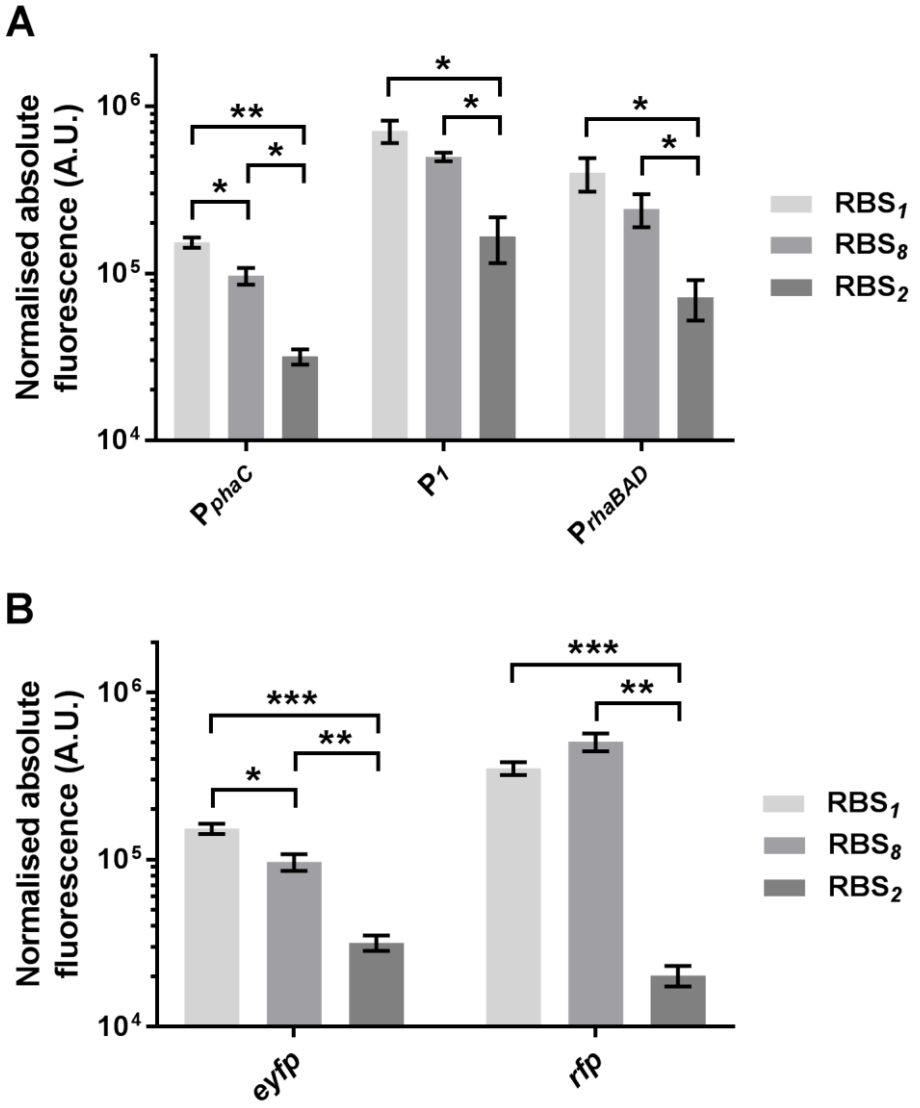
Supplementary Figure S3. Correlation between promoter activities, measured as normalised fluorescence arbitrary units (A.U.) using either GFP as reporter in previous study (6) and EYFP in this study. Core promoters derived from bacteriophage T5 and *C. necator* H16 (promoters P_{T5}, P_{j5}, P_{h207}, P_{h25} and P_{H16_B1772} (6)) were used in analysis. In this study they comprise core part of promoters P_1, P_4, P_5, P_6, and P_2, respectively.



Supplementary Figure S4. Consumption of acrylate in *C. necator*. Acrylate was added at time zero. Consumption of acrylate and fructose was monitored using HPLC-UV in supernatant over the time course of 48 hours. The means of three biological replicates are presented. Error bars are too small to be visible.



Supplementary Figure S5. Induction dynamics for the L-arabinose-inducible system lacking the stem-loop structure. Normalised absolute fluorescence of *C. necator* cells in exponential growth phase six hours after L-arabinose addition. The standard deviation of three biological replicates is illustrated as band.



Supplementary Figure S6. Effect of sequence content on the strength of RBS. Normalised absolute fluorescence determined using plasmid constructs with promoters P_{phaC}, (P_{0_1}), P_1, and P_{rhaBAD} (A), and reporter genes *eyfp* and *rfp* (B) located upstream and downstream, respectively, of RBS_1, RBS_8 and RBS_2. Error bars represent standard deviation of three biological replicas. Asterisks indicate statistically significant difference between RBS strengths: *$p < 0.01$; **$p < 0.001$; and ***$p < 0.0001$ (unpaired t-test).

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