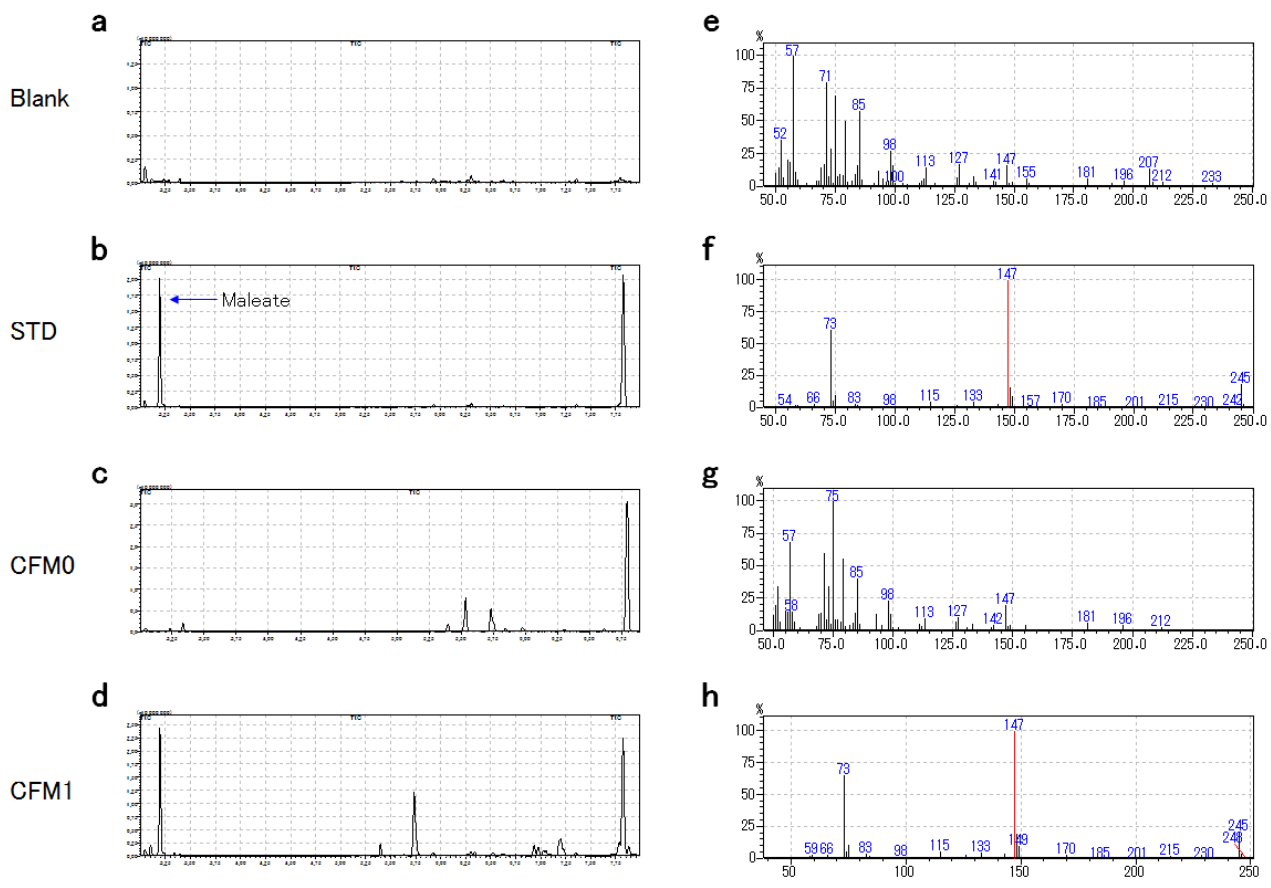
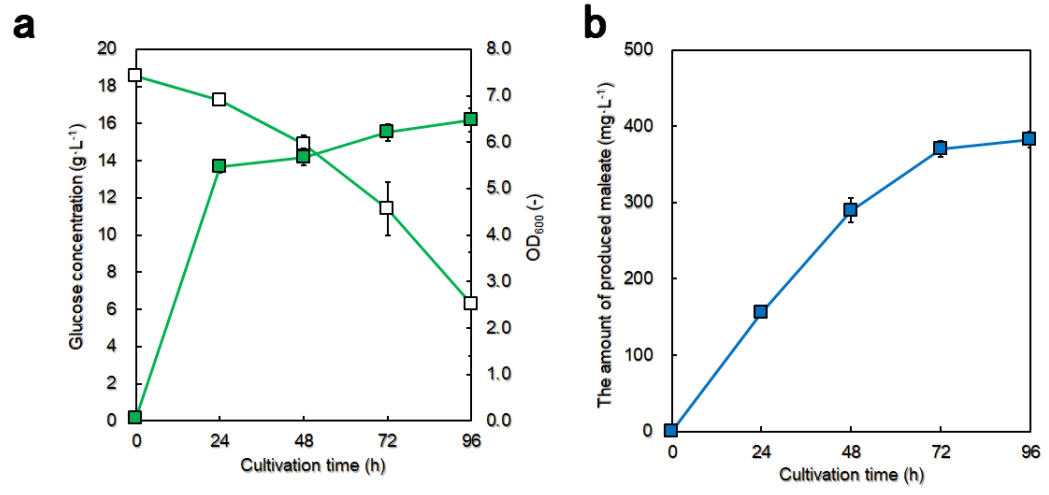
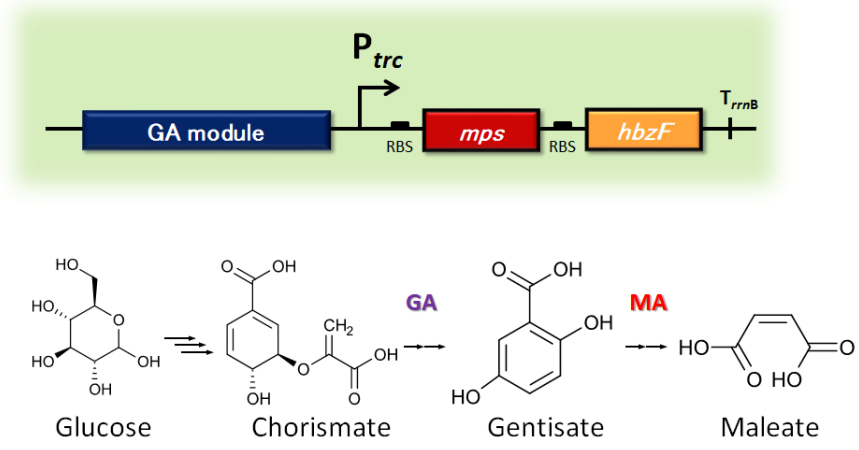


Supplementary Figure 1 | Maps of the plasmids constructed in this study. (a)

pTrcHisB carrying the GA module (pTcgl3026, pT3hb6h, and pTxlnD). **(b)** pZA23MCS carrying the MA module (pZA23psal, pZA23ppu1, pZA23palc, pZA23rsp, and pZA23ppu2). **(c)** pTrcHisB carrying the GA and MA modules (pT2c101-104 and pT2c201-204). **(d)** pTrcHisB carrying the MAGA or GAMA module (pT2t01 or pT2t02). **(e)** pSAK carrying the Hyg5 cassette or GA module (pS09 or pS10). **(f)** pZA23MCS carrying the Hyg5 cassette (pA09).

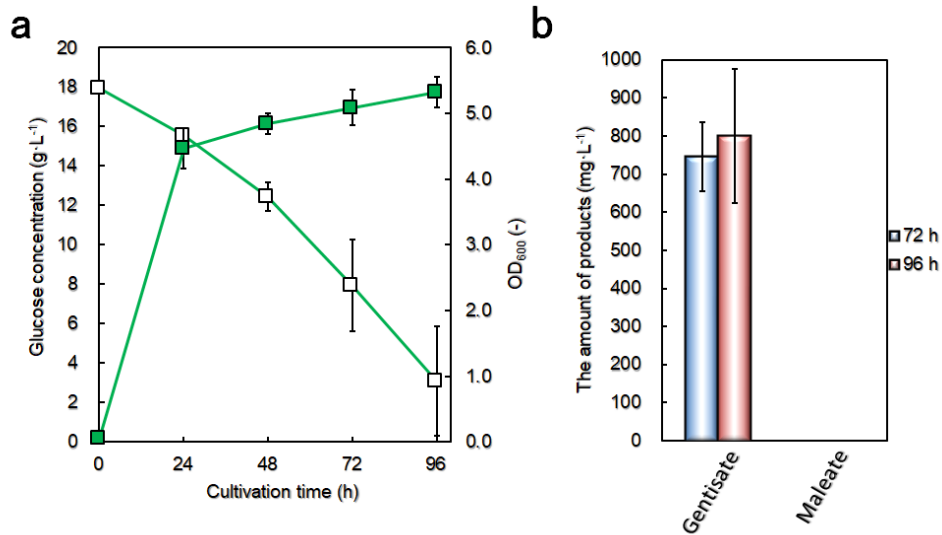
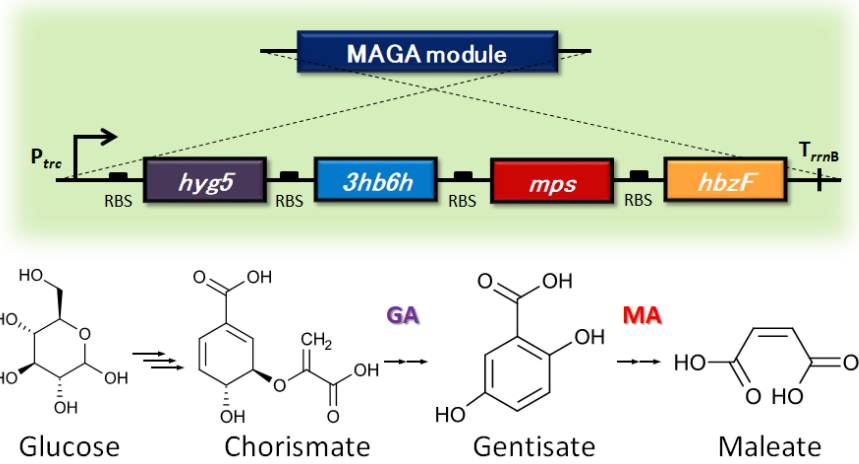


Supplementary Figure 2 | Confirmation of maleate production by gas chromatography–mass spectrometry analysis. (a–d) GC–MS chromatograms of maleate production. (e–h) MS spectra at 3.2 min in the GC–MS analysis. Each pair of a and e, b and f, c and g, and d and h presents the results for a blank, standard sample, CFM0, and CFM1, respectively.



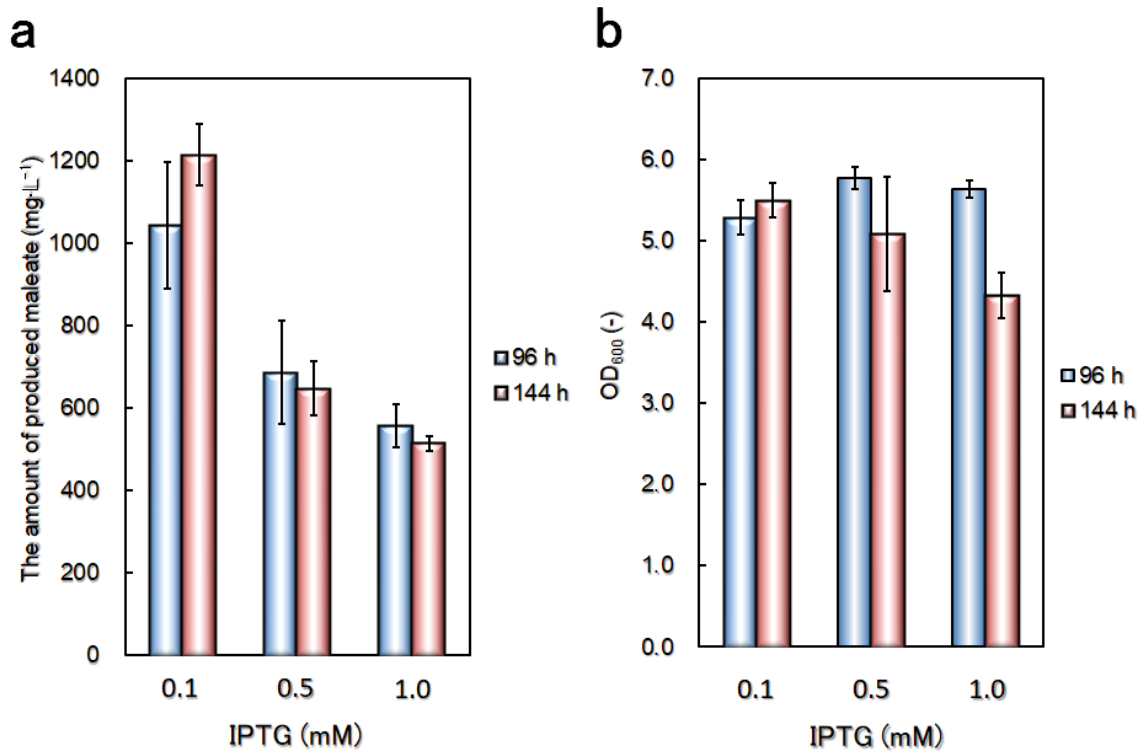
Supplementary Figure 3 | Culture profile of CFM9 during maleate production.

Time courses of (a) the glucose concentration (open symbols) and OD₆₀₀ (filled symbols), and (b) the amount of produced maleate. Data are presented as the mean ± standard deviation of three independent experiments ($n = 3$).

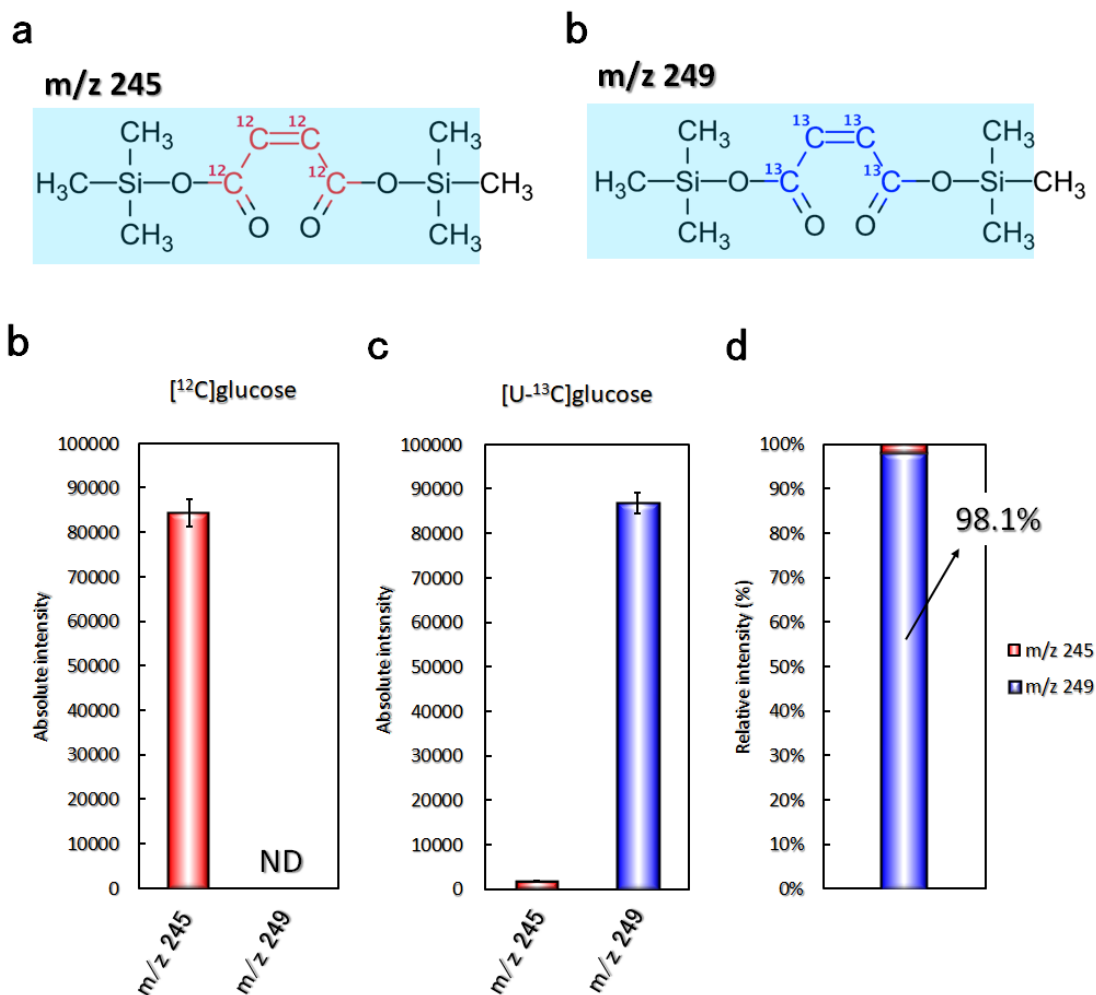


Supplementary Figure 4 | Culture profile of CFMt2 during maleate production.

Time courses of **(a)** the glucose concentration (open symbols) and OD₆₀₀ (filled symbols), and **(b)** the amounts of produced maleate and gentisate. Data are presented as the mean ± standard deviation of three independent experiments ($n = 3$).

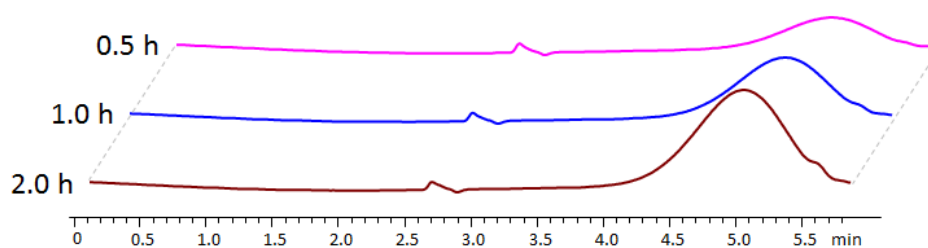


Supplementary Figure 5 | Effects of the concentration of β -D-1-thiogalactopyranoside (IPTG). (a) Amounts of maleate produced by CFMt1 after 96 and 144 h of cultivation. (b) OD₆₀₀ values of CFMt1 after 96 and 144 h of cultivation. Data are presented as the mean \pm standard deviation of three independent experiments ($n = 3$).

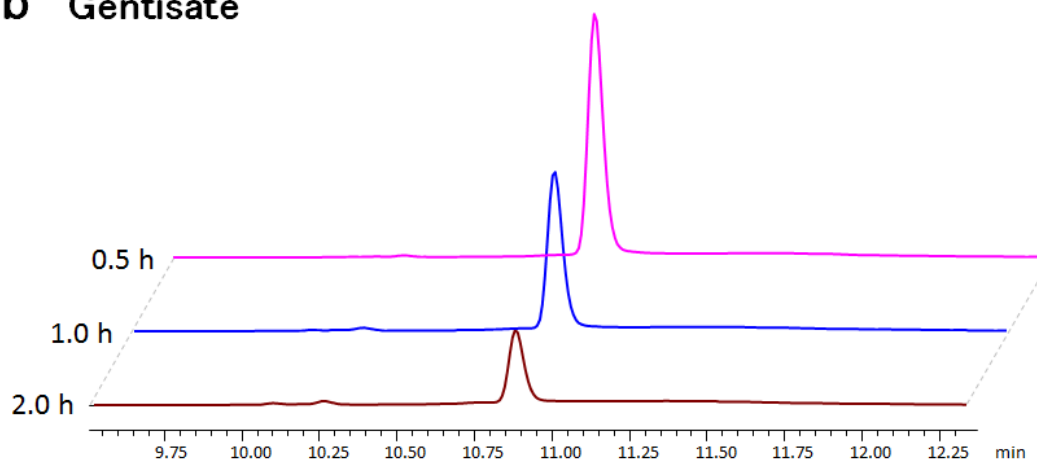


Supplementary Figure 6 | Labeling pattern of maleate produced by CMtS101 cultured using [¹²C]glucose or [U-¹³C]glucose as the carbon source. (a) Structure of maleate–bis(trimethylsilyl) ester derivative and m/z of the demethylated product. (b) Absolute intensity of m/z = 245 and 249 in the case of using [¹²C]glucose. (c) Absolute and (d) relative intensity of m/z = 245 and 249 in the case of using [U-¹³C]glucose. Data are presented as the mean ± standard deviation of three independent experiments (*n* = 3).

a Malelpyruvate



b Gentisate



Supplementary Figure 7 | Time-course high-performance liquid chromatography chromatograms of (a) malelpyruvate formation and (b) gentisate consumption in enzymatic conversion of gentisate to malelpyruvate.

Supplementary Table 1. Summary of the amounts of consumed glucose and yields of produced gentisate in cultures of CFG1 -3.
Data are presented as the mean \pm standard deviation of three independent experiments ($n = 3$).

	Strains		
	CFG1	CFG2	CFG3
Consumed glucose ($\text{g}\cdot\text{L}^{-1}$)	5.7 ± 0.8	5.9 ± 0.5	17.9 ± 0.6
Gentisate yield ($\text{mol}\cdot\text{mol}^{-1}$)	0.165 ± 0.022	0.130 ± 0.002	0.022 ± 0.007

Supplementary Table 2. Summary of the amounts of consumed glucose and yields of produced maleate in eight transformants.
 Data are presented as the mean \pm standard deviation of three independent experiments ($n = 3$).

	Strains							
	CFM1	CFM2	CFM3	CFM4	CFM5	CFM6	CFM7	CFM8
Consumed glucose ($\text{g}\cdot\text{L}^{-1}$)	3.4 ± 0.1	4.8 ± 1.3	7.8 ± 0.6	6.0 ± 0.1	2.8 ± 0.1	1.2 ± 0.2	0.9 ± 0.2	1.1 ± 0.1
Maleate yield ($\text{mol}\cdot\text{mol}^{-1}$)	$0.189 \pm$ 0.018	$0.477 \pm$ 0.0093	$0.0868 \pm$ 0.0085	$0.0842 \pm$ 0.0086	$0.117 \pm$ 0.010	$0.0896 \pm$ 0.0401	$0.0749 \pm$ 0.0014	$0.0528 \pm$ 0.0023

Supplementary Table 3. Amounts of produced organic acids. Data are presented as the mean \pm standard deviation of three independent experiments ($n = 3$).

Strains	Time (h)	Produced by-products (mg·L ⁻¹)				
		Acetate	Pyruvate	Lactate	Formate	Malate
CFM00	24	2,890 \pm 310	21 \pm 21	ND	ND	31 \pm 4
	48	4,750 \pm 330	76 \pm 15	36 \pm 3	ND	19 \pm 6
	72	5,370 \pm 89	157 \pm 4	126 \pm 10	ND	ND
CFM01	24	ND	ND	ND	ND	ND
	48	ND	ND	ND	ND	ND
	72	ND	ND	ND	ND	ND
CFM1	72	ND	ND	ND	14 \pm 3	ND
	94	17 \pm 30	ND	ND	17 \pm 5	ND
	144	540 \pm 440	ND	ND	20 \pm 5	ND
CFMt1	72	ND	ND	ND	5 \pm 5	ND
	94	40 \pm 35	ND	ND	11 \pm 3	ND
	144	640 \pm 400	ND	ND	18 \pm 6	ND
CFMtS09	24	ND	ND	ND	ND	ND
	48	ND	ND	ND	ND	ND
	72	82 \pm 4	ND	ND	ND	ND
CFMtS10	24	ND	ND	ND	ND	ND
	48	16 \pm 27	ND	ND	ND	ND
	72	74 \pm 11	ND	ND	ND	ND
CFMtS091	24	ND	ND	ND	ND	ND
	48	ND	ND	ND	ND	ND
	72	ND	ND	ND	ND	ND
CFMtS101	24	ND	ND	ND	ND	ND

48	ND	ND	ND	ND	ND
72	ND	ND	ND	ND	ND

ND, not
detected.

Supplementary Table 4. Strains, plasmids, and transformants used in this study

Strains or plasmids	Genotype	Source or reference
Strains		
NovaBlue	<i>endA1 hsdR17</i> (rK12 ⁻ mK12 ⁺) <i>supE44 thi-I gyrA96 relA1 lac recA1/F'</i> [<i>proAB</i> ⁺ <i>lacI</i> ^q <i>ZΔM15::Tn10</i> (Tet ^r)]; used for gene cloning	Novagen
BL21 (DE3) pLysS	F ⁻ <i>ompT hsdS</i> (<i>r_B⁻ m_B⁻) <i>gal dcm λ</i>(DE3) pLysS (Cam^r) (<i>λ</i>(DE3): <i>lacI</i>, <i>lacUV5-T7 gene 1, ind1, sam7, nin5</i>); used for protein expression</i>	TaKaRa Bio
ATCC 31882	L-Phenylalanine-overproducing strain	American Type Culture Collection
CFT3	ATCC31882 <i>ptsHI::P_{A1lacO-1}-Glk-GalPΔpykFΔpykA</i>	[1]
CFT5	ATCC31882 <i>ptsHI::P_{A1lacO-1}-Glk-GalPΔpykFΔpykAΔpheΔtyrA</i>	[1]
CFG0	CFT5 harboring pTrcHisB	This study
CFG1	CFT5 harboring pTcgl3026	This study
CFG2	CFT5 harboring pT3hb6h	This study
CFG3	CFT5 harboring pTxlnD	This study
CFMP0	CFT5 harboring pZA23MCS	This study
CFMP1	CFT5 harboring pZA23psal	This study
CFMP2	CFT5 harboring pZA23ppu1	This study
CFMP3	CFT5 harboring pZA23palc	This study
CFMP4	CFT5 harboring pZA23rsp	This study
CFMP5	CFT5 harboring pZA23ppu2	This study
CFM1	CFT5 harboring pT2c101	This study
CFM2	CFT5 harboring pT2c102	This study
CFM3	CFT5 harboring pT2c103	This study
CFM4	CFT5 harboring pT2c104	This study

CFM5	CFT5 harboring pT2c201	This study
CFM6	CFT5 harboring pT2c202	This study
CFM7	CFT5 harboring pT2c203	This study
CFM8	CFT5 harboring pT2c204	This study
CFM9	CFT5 harboring pT2c301	This study
CFMt1	CFT5 harboring pT2t01	This study
CFMt2	CFT5 harboring pT2t02	This study
CMtS00	CFMt1 harboring pSAK	This study
CMtS01	CFMt1 harboring pS01	This study
CMtS02	CFMt1 harboring pS02	This study
CMtS03	CFMt1 harboring pS03	This study
CMtS04	CFMt1 harboring pS04	This study
CMtS05	CFMt1 harboring pS05	This study
CMtS06	CFMt1 harboring pS06	This study
CMtS07	CFMt1 harboring pS07	This study
CMtS08	CFMt1 harboring pS08	This study
CMtS09	CFMt1 harboring pS09	This study
CMtS10	CFMt1 harboring pS10	This study
CMtS091	CMtS09 harboring pA09	This study
CMtS101	CMtS10 harboring pA09	This study
BL21MP	BL21 (DE3) pLysS harboring pETM1	This study
Plasmids		
pTrcHis B	P _{trc} , pBR322 ori, Amp ^r	Life Technologies
pZA23MCS	P _{AlacO1} , p15A ori, Km ^r	Expressys
pZA33luc	P _{AlacO1} , luciferase gene, p15A ori, Cm ^r	Expressys

pZS4Int-laci	<i>lacI^d</i> , SC101 ori, Spec ^r	Expressys
pSAK	P _{AlacO1} , SC101 ori, Cm ^r	This study
pET-22b(+)	P _{T7} , pBR322 ori, Amp ^r	Novagen
pTcgl3026	pTrcHis B containing <i>hyg5</i> from <i>Streptomyces hygroscopicus</i> ATCC 29253 and <i>cgl3026</i> from <i>Corynebacterium glutamicum</i>	This study
pT3hb6h	pTrcHis B containing <i>hyg5</i> and <i>3hb6h</i> from <i>Rhodococcus jostii</i> RHA1	This study
pTxlnD	pTrcHis B containing <i>hyg5</i> and <i>xlnD</i> from <i>Pseudomonas alcaligenes</i> NCIMB 9867	This study
pZA23hbzF	pZA23MCS containing <i>hbzF</i> from <i>P. alcaligenes</i> NCIMB 9867	This study
pZA23psal	pZA23MCS containing <i>mps0</i> from <i>Pseudaminobacter salicylatoxidans</i> and <i>hbzF</i>	This study
pZA23ppu1	pZA23MCS containing <i>sgp1</i> from <i>Pseudomonas putida</i> and <i>hbzF</i>	This study
pZA23palc	pZA23MCS containing <i>mps1</i> from <i>P. alcaligenes</i> NCIMB 9867 and <i>hbzF</i>	This study
pZA23rsp	pZA23MCS containing <i>mps2</i> from <i>Rhodococcus</i> sp. strain NCIMB 12038 and <i>hbzF</i>	This study
pZA23ppu2	pZA23MCS containing <i>mps3</i> from <i>P. putida</i> and <i>hbzF</i>	This study
pT2c101	pT3hb6h containing <i>mps2</i> with P _{AlacO1} promoter	This study
pT2c102	pT3hb6h containing <i>mps3</i> with P _{AlacO1} promoter	This study
pT2c103	pT3hb6h containing <i>mps1</i> with P _{AlacO1} promoter	This study
pT2c104	pT3hb6h containing <i>sgp1</i> with P _{AlacO1} promoter	This study
pT2c201	pTcgl3026 containing <i>mps2</i> with P _{AlacO1} promoter	This study
pT2c202	pTcgl3026 containing <i>mps3</i> with P _{AlacO1} promoter	This study
pT2c203	pTcgl3026 containing <i>mps1</i> with P _{AlacO1} promoter	This study
pT2c204	pTcgl3026 containing <i>sgp1</i> with P _{AlacO1} promoter	This study

pTmpsR	pTrcHis B containing <i>mps2</i> and <i>hbzF</i>	This study
pT2c301	pT3hb6h containing <i>mps2</i> and <i>hbzF</i> with P _{trc} promoter	This study
pZA01	pZA23MCS containing <i>mps2</i> , <i>hbzF</i> , <i>hyg5</i> , and <i>3hb6h</i> in tandem	This study
pT2t01	pTrcHis B containing <i>mps2</i> , <i>hbzF</i> , <i>hyg5</i> , and <i>3hb6h</i> in tandem	This study
pT2t02	pTrcHis B containing <i>hyg5</i> , <i>3hb6h</i> , <i>mps2</i> , and <i>hbzF</i> in tandem	This study
pS01	pSAK containing <i>aroF^{br}</i> with P _{AlacO1} promoter	This study
pS02	pSAK containing <i>aroG^{br}</i> with P _{AlacO1} promoter	This study
pS03	pSAK containing <i>tktA</i> with P _{AlacO1} promoter	This study
pS04	pSAK containing <i>ppc</i> with P _{AlacO1} promoter	This study
pS05	pSAK containing <i>maeB</i> with P _{AlacO1} promoter	This study
pS06	pSAK containing <i>ppsA</i> with P _{AlacO1} promoter	This study
pS07	pSAK containing <i>glk</i> with P _{AlacO1} promoter	This study
pS08	pSAK containing <i>galP</i> with P _{AlacO1} promoter	This study
pS09	pSAK containing <i>hyg5</i> with P _{trc} promoter	This study
pS10	pSAK containing <i>hyg5</i> and <i>3hb6h</i> in tandem under P _{trc} promoter	This study
pA09	pZA23MCS containing <i>hyg5</i> with P _{trc} promoter	This study
pETM1	pET-22b(+) containing <i>mps2</i>	This study

Abbreviations: Amp, ampicillin; Km, kanamycin; Cm, chloramphenicol; Spec, spectinomycin.

Supplementary Table 5. Oligonucleotide primers used in this study

Oligonucleotide primers	Sequence
3hb6h_f	5'-ATCTGCAGCTGGTACTTAAAGAGGTATATAATGAGCAATCTGCAGGATGC-3'
3hb6h_r	5'-ATTCCCATATGGTACTTAGCTTGCACGATCGCTGC-3'
xlnD_f	5'-ATCTGCAGCTGGTACTTAAAGAGGTATATAATGCACAACAACATTCTGAT-3'
xlnD_r	5'-ATTCCCATATGGTACTTATTTGTATTTCTCGACGG-3'
plac_to_ptrc_f	5'-CTCCTGAGTAGGACACGAGGCCCTTTCGTCTTCAC-3'
plac_to_ptrc_r	5'-CGCTCCCGGCGGATTTCTAGGGCGGGCGGATTTGTC-3'
inv_ptrc_840_f	5'-AATCCGCCGGGAGCGGATTTGAACG-3'
inv_ptrc_840_r	5'-TGTCCTACTCAGGAGAGCGTTCACC-3'
mpr_hbz_ptrc_f	5'-TATCGAATTCCTGCACGACCGGAATTATCGATTAA-3'
mpr_hbz_ptrc_r	5'-ATCCCCCGGGCTGCAATCTGTATCAGGCTGAAAAT-3'
inv_ptrc_f	5'-TATCGAATTCCTGCACGACCGGAATTATCGATTAA-3'
inv_ptrc_r	5'-ATCCCCCGGGCTGCAATCTGTATCAGGCTGAAAAT-3'
ptrc_to_ptrc_840_f	5'-CTCCTGAGTAGGACATGTTGACAATTAATCATCCG-3'
ptrc_to_ptrc_840_r	5'-CGCTCCCGGCGGATTAAGGCCAGTCTTTCGACTG-3'
ptrc_rbs_pz_psti_f	5'-TATCGAATTCCTGCACGACCGGAATTATCGATTAA-3'
ptrc_rbs_pz_psti_r	5'-ATCCCCCGGGCTGCAATCTGTATCAGGCTGAAAAT-3'
inv_pZ_ptsi_f	5'-TGCAGCCCCGGGGGATCCCAT-3'
inv_pZ_ptsi_r	5'-TGCAGGAATTCGATATCTTA-3'
mpr_to_ptrc_f	5'-CATCATCATCATGGTATGACCGCAACCGAACCGCA-3'
3hb6h_to_ptrc_r	5'-TCTCGAGCTCGGATCTTAGCTTGCACGATCGCTGC-3'
mpr_hbz_to_pt_hind_f	5'-CATATGGGAATTCGAGCGGATAACAATTCACACA-3'
mpr_hbz_to_pt_hind_r	5'-CCGCCAAAACAGCCAACGCGTACCATGGGATCCCC-3'
cmr_f	5'-GAGCTCGATATCAAATTACG-3'

cmr_r	5'-GTGAAGACGAAAGGGCCTCG-3'
sc101_f	5'-CCTAGGGTACGGGTTTTGCT-3'
sc101_r	5'-TTTGATATCGAGCTCCGCTTGGACTCCTGTTGATA-3'
palac_f	5'-CCCTTTCGTCTTCACAAATTTATCAAAAAGAGTGT-3'
palac_r	5'-AACCCGTACCCTAGGGTCTAGGGCGGCGGATTTGT-3'
arof_f	5'-AGAGGAGAAAGGTACATGAATTATCAGAACGACGA-3'
arof_r	5'-GGGGGGGCCCCGGTACTTACCCGCGACGCGCTTTTA-3'
arog_f	5'-AGAGGAGAAAGGTACATGCAAAAAGACGCGCTGAA-3'
arog_r	5'-GGGGGGGCCCCGGTACTTAAGCCACGCGAGCCGTCA-3'
tkta_f	5'-AGAGGAGAAAGGTACATGTCCTCACGTAAAGAGCT-3'
tkta_r	5'-GGGGGGGCCCCGGTACTTACAGCAGTTCTTTTGCTT-3'
ppc_f	5'-AGAGGAGAAAGGTACATGAACGAACAATATTCCGC-3'
ppc_r	5'-GGGGGGGCCCCGGTACTTAGCCGGTATTACGCATAC-3'
maeb_f	5'-AGAGGAGAAAGGTACATGGATGACCAGTTAAAACA-3'
maeb_r	5'-GGGGGGGCCCCGGTACTTACAGCGGTTGGGTTTGCG-3'
ppsa_f	5'-AGAGGAGAAAGGTACATGTCCAACAATGGCTCGTC -3'
ppsa_r	5'-GGGGGGGCCCCGGTACTTATTTCTTCAGTTCAGCCA-3'
glk_f	5'-AGAGGAGAAAGGTACATGACAAAGTATGCATTAGT-3'
glk_r	5'-GGGGGGGCCCCGGTACTTACAGAATGTGACCTAAGG-3'
galp_f	5'-AGAGGAGAAAGGTACATGCCTGACGCTAAAAACA-3'
galp_r	5'-GGGGGGGCCCCGGTACTTAATCGTGAGCGCCTATTT-3'
ptrc_psak_r	5'-AGTCCAAGCGGAGCTTGTTGACAATTAATCATCCG-3'
ptrc_psak_f	5'-ATTTGATATCGAGCTAAGGCCAGTCTTTCGACTG-3'
ptrc_pza23_f	5'-TATCATGACATTAAGTGTGACAATTAATCATCCG-3'
ptrc_pza23_r	5'-AGTCAGTGAGCGAGGAAAGGCCAGTCTTTCGACTG-3'
inv_pza23_f	5'-CCTCGCTCACTGACTCGCTA-3'

inv_pza23_r

5'-GTTAATGTCATGATAATAAT-3'

mps2_pet22_f

5'-AGAAGGAGATATACAATGACCGCAACCGAACCGCA-3'

mps2_pet22_r

5'-CTCGAGTGCGGCCGCTGCACCTTCGCTAATATAGG-3'

Supplementary Table 6. Summary of proteins for maleate production.

Name	Species	NCBI accession	EC number
Hyg5	<i>Streptomyces hygroscopicus</i>	O30478.1	4.1.3.45
Cgl3026	<i>Corynebacterium glutamicum</i> ATCC 13032	Q8NLB6.1	1.14.13.24
3HB6H	<i>Rhodococcus jostii</i> RHA1	5HYM_A	1.14.13.24
XlnD	<i>Pseudomonas alcaligenes</i> NCIMB 9867	AAG39455.1	1.14.13.24
Mps0	<i>Pseudaminobacter salicylatoxidans</i>	AAQ91293	1.13.11.4
Sgp1	<i>Pseudomonas putida</i>	ACO92375.1	1.13.11.4
Mps1	<i>Pseudomonas alcaligenes</i> NCIMB 9867	AF173167_1	1.13.11.4
Mps2	<i>Rhodococcus</i> sp. strain NCIMB 12038	ADT78164.1	1.13.11.4
Mps3	<i>Pseudomonas putida</i>	WP_047604952.1	1.13.11.4
HbzF	<i>Pseudomonas alcaligenes</i> NCIMB 9867	ABD64512.1	3.7.1.23

Supplementary Note 1

Sequence of *3hb6b*:

ATGAGCAATCTGCAGGATGCCCGTATTATCATTGCCGGTGGTGGTATTGGTGG
TGCAGCAAATGCACTGGCACTGGCCCA GAAAGGTGCAAATGTTACCCTGTTT
GAACGTGCAAGCGAATTTGGTGAAGTTGGTGCA GGTCTGCA GGTGGTCCG
CATGGTGCACGTATTCTGGATA GCTGGGGTGTCTGGATGATGTTCTGAGCCG
TGCATTTCTGCCGAAAAATATCGTTTTTCGTGATGCCATTACCGCAGAAGTTC
TGACCAA AATTGATCTGGGTAGCGAATTCGTGGTCTGTTATGGTGGTCCGTAT
TTTGTACCATCGTAGCGATCTGCATGCAACCCTGGTTGATGCAGCACGTGC
AGCCGGTGCAGAACTGCATACCGGTGTTACCGTTACCGATGTTATTACCGAAG
GTGATAAAGCAATTGTGAGCACCGATGATGGTCTGACCCATGAAGCAGATATT
GCACTGGGTATGGATGGTCTGAAAA GCCGTCTGCGTGAAAAAATCAGCGGTG
ATGAACCGTTAGCAGCGGTTATGCA GCATATCGTGGCACCAACCCGTATCGT
GATGTTGAACTGGATGAAGATATTGAAGATGTGGTGGGTTATATTGGTCCGCG
TTGTCATTTTATTCAGTATCCGCTGCGTGGTGGTCAAATGCTGAATCAGGTTG
CAGTTTTTGAAAGTCCGGGATTCAAAAACGGCATTGAAAATTGGGGTGGTCC
GGAAGAACTGGAACAGGCATATGCACATTGCCATGAAAATGTTCTGTCGCGGT
ATTGATTATCTGTGGAAAGATCGTTGGTGGCCGATGTATGATCGTGAACCGAT
TGAAA AACTGGGTTGATGGTCTGCATGATTCTGCTGGGTGATGCCGCACATCCG
CCTCTGCAGTATCTGGCAAGCGGTGCAGTTATGGCAATCGAAGATGCAAAAT
GTCTGGCAGATTATGCAGCCGAA GATTTTAGCACCGGTGGTAATAGCGCATGG
CCTCAGATTCTGAAAGAAGTTAATACCGAACGCGCACCGCGTTGTAATCGTAT
TCTGACCACCGGTCGTATGTGGGGTGA ACTGTGGCATCTGGATGGCACCGCA

CGTATTGCACGTAATGAACTGTTTCGTACCCGTGATACCAGCAGCTATAAATA
CACCGATTGGCTGTGGGGTTATAGCAGCGATCGTGCAAGCTAA

Sequence of *xlnD*:

ATGCACAACAACATTCTGATTGCCGGTGCA GGTATTGGTGGTCTGAGCGCAG
CACTGGGTCTGGCACGTAAAGGTATGCGTAGCATTGTTCTGGAAAAAGCACC
GGA ACTGGGTGAAATTGGTGCCGGTATTCA GCTGGCACC GAATGCATATCAT
GCACTGGATGCCCTGGGTATTGGCGAAGTTGCACGTCAGACCGGTGTTTCATG
TTGATAAACTGCTGTGGATGGATGGTATGACCGATAAAGAAATTGCAAGCGTT
CCGCTGGCAAATCGTTTTTCGTGAATTTTTTGGTAATCCGTATGCCGTTATTCAC
CGTGCAGATTTTCATGGTCTGCTGGTTGAA GCATGTCATAAAACCGGTCTGGT
GGAAGTTCGTACCAATGCCGAAGTTGTTGATTATGAAA ACTTTCCGGATCGTG
TTGAAGCCATTCTGCATGATGGTAGCTGTATTAATGGTGCA GTTCTGGTTGGT
GCAGATGGTCTGTGGTCAAATGTTTCGTCAGAAA GTTATTGGTGATGGTGATCC
GCGTGTTAGCGGTCATAACAACCTATCGTAGCGTTATTCCGGCAGAA GATATGC
CGGAAGAACTGCGTTGGAATATGAGCACCGCATGGGCA GGC GAA GGTTGTC
ATATGGTTCATTATCCGCTGAAAGGTGGCAAAGTTTTTAATCTGGTTCTGACC
AGCAATAGCGGTGCAAGCGAACC GGAAGCCGGTGTCCGGTTACCACCGAT
GAAGTTTTTGAAAAATTCAA AACCATGAAACGTCGTCCGACCAGCCTGATTC
ATAAAGGTAATAATTGGAAACGTTGGGTGCTGTGCGATCGTGATCCGCTGCCG
AATTGGGTTGATGGTCGTGTTACCCTGCTGGGTGATGCAGCACATCCGATGAT
GCAGTATATGGCACAGGGTGCAAGCATGGCAATTGAA GATGCA GTTTGTCTG
GCATTTGAACTGGGTCTGTAATGGATCCGGTTAGCGCACTGAAAAAATACA
ATCGTGCACGTTTTGCACGTACCGCACGTGTTTCAGACCTATAGCCGTTATGCA

AGCGATTTTATCTATCATGCAAAAGGTGGTGCAGCAGCAATGCGTAATGAACT
GATGGGTGGCATGACCCCGACC GATTTTTTCCAGTGGATTAATTGGCTGTATG
GCAAAGAAACCGTCGAGAAATACAAA TAA

Sequence of *cgl3026*:

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CAGCAGAATTTAAAGAAGTTGGCGCAGGTCTGCAGATTGGTCCGCATGGTTG
GCGTATGCTGGAAAGCTGGGGTCTGCTGGATCAGATTGTTGTTGCAGGTTATC
TGCCGGAAGATATGCAGTTTCGTGATGCAGTTAATCGTGAAACCATTCTGACC
ATGCGTTTTGATGAAGAATTCAGCAGCATTATGGTGGTCGTTATCTGGTTATT
CATCGTAGCGATCTGCTGAATATCCTGGTTACCAATGCCGAAGCAGCGGGTGC
AAA ACTGCACAATGGTGTCTGGTTACCGATAGCCGTACCGTTGATGGTGGCA
TTGAA GTTGATATTGAAAGCAGCATCAATAAAGGCGAGGATAACAAAACCT
GCTGGTTGATGCATTTCTGGCATTGATGGTATTCATAGCGTGATGCGTAAAA
AACTGGTGGATGATGCACCGGTTGCAAGCAGCTATGTTGCATATCGTGGCAC
CAGCAA ACTGGCCGAAGATGCAGAAATGAAAGATCTGAAAAGCGTGATTGG
TTATATTGGACCGCATGTGCATTTTATTCA GTATCCGCTGCGTGGCGGTGAACT
GCTGAATCAGGTTGCAGTTTTT GAAAGCCAGCGCTATCTGGATGGTCGTACC
GCAGGC GATATTCCGGAA GATTGGGGTAATCCGGAAGAACTGGATCGTG CAT
ATAATCATTGTGATCCGTTTATTCAGGATCGTCTGGATAACCCTGTGGCGTAATA
ATTGGTGGCAGATGAGCGATCGTGAACCGCTGGAAAATTGGCGTATTGGTCG
CATGCTGCTGCTGGGAGATGCAGCACATGCACCGCTGCAGTATCTGGCAAGC

GGTGCAGTTATGGCAATGGAA GATGCGGAAGCA GTTGC ACTGTTTGCAGCAG
ATGCAGCCCGTGCAGGTAATCTGGATTGGGAA GAAGTTCTGGCAGAAGTTGA
AGCAGAACGTCGTCCGC GTTGTAGCCGTATTCAGACCGTTGGTCGTTTTTGG
GGTGA ACTGTGGCATGTTGAA GGCACCGCACGTCTGATTCGTAATGAAGTTT
TTCGT CAGGCAGATCGTAATGGCTGGTTTATCTATGCCGATTGGCTGTGGGGT
TATGATGCAAGCAAACGTGCACATATTGCAAATCCGGA ACTGGGTGAAATGC
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CATATGGGAAT

Sequence of *hbzF*:

GTCGACGGTATCGATTCATTAAGAGGAGAAAGGT ATGAAAATCTGCCGCT
TTAATGAAAATCGTCTGGGTGTTGTTGATGGTGATGAA GTTCTGGATGTTACC
GAAGCACTGAGCGTTCTGCCGAGCTATGAATATCCGCTGCCTGGTTATGATCC
GCTGATTAAACATCTGGATGCACTGCTGGCACGTATTGAAACCCTGATTAAAG
ATGCACCGCGTATTCTGCTGGCAGATGTTCTGCTGCTGAGTCCGGTTGCAAAT
CCGGGTAAAATCATTGCAGCACCGATTA ACTATAACCCGTCATCTGCAAGAAGT
GCTGGCCGATAGCGCAATTAACAATGGTGTTGCAAGCTTTACCCAGCATATCA
AAAAAAGCGGTCTGTTTCTGAAAGCAAATAGCAGCCTGGCTGGTGCCGGTG
AAGGTGTTGCACTGAGCCATCAGGATCGTCGTAATGATCATGAA GTTGA ACT
GGCCATTGTGATTGGTAAAACCGCACGTAATGTTCCGCGTGAAAAAAGCCTG
GAATATGTTGCAGGTTATTGCATTGGTATTGATATGACCGTTCTGGTCCGGAA
GAACGTAGCTTTCGTAAAAGTCCGGATAGCTATAACCATCTGGGTCCGTGGCT
GGTTACCCGTGATGAAATTGATAGTCCGGGTGAACTGCAGATGAGCCTGAAA
GTTAATGGTGAAGTTCGTCAGAATGCAAATACCA GCGATCTGATTCTGGGTGT

GGAAGAACTGGTTGAATTTGCAAGCAGCTTTTATACCCTGCATCCGGGTGAT
GTGATTATTAGCGGTACACCGGAA GGTGTGGGTCCGGTTAATCCTGGTGATGC
CATGCTGGCGGAAATTGAACGTATTGGCACCATGACCATTGCAATTCGTAGCG
TTGACTACAAGGATGACGATGACAAGTAA GATATCGAATTCCTG

Sequence of *mps0*:

ATTAAAGAGGAGAAAGGTACCATGCAGAACGAAAACTGGATCATGAAAGC
GTTACCCAGGCAATGCAGCCGAAAGATACACCGGAACTGCGTGC ACTGTATA
AAAGCTTTGAAGAA GAAAGCATTATTCCGCTGTGGACACAGCTGGGTGATCT
GATGCCGATTCATCCGAAAAGCAAAGCA GTTCCGCATGTTTGGAAATGGTCA
ACCCTGCTGCGTCTGGCACGTAAAAGCGGTGAACTGGTTCGGTTGGTCGTG
GTGGTGAACGTCGTGCACTGGGTCTGGCAAATCCGGGTCTGGGTGGTAATGC
ATATATTAGCCCGACCATGTGGGCAGGTATTCAGTATCTGGGTCCGCGTGAAA
CCGCACCGGAACATCGTCATAGCCAGAATGCATTTCTGTTTTGTTGTTGAAGGT
GAAGGTGTTTGGACCGTTGTTAATGGTGATCCGGTTCGTATGAGCCGTGGTGA
TCTGCTGCTGACACCGGGTTGGTGTTCATGGTCATCATAATGATACCGATC
AGCCGATGGCATGGATTGATGGTCTGGATATTCGGTTTAGCCAGCA GATGGAT
GTTGGCTTTTTTGAATTTGGTAGCGATCGTGTTACCGATTATGCAACCCCGAAT
TTTAGCCGTGGCGAACGTCTGTGGTGTATCCTGGTCTGCGTCCGCTGAGCG
GTCTGCAGAAATACCGTTGCAAGCCCGATTGGTGCATATCGTTGGGAATTTACC
GATCGTGCCCTGACCGAACAGCTGCTGCTGGAAGATGAAGGTCA GCCTGCA
ACCGTTGCACCGGGTTCATGCAGCAATTCGTTATGTTAATCCGACCACAGGTG
GTGATGTTATGCCGACCCTGCGTTGTGAATTCATCGTCTGCGTGCAGGCACC
GAAACCGCAACCCGTAATGAAGTTGGTAGCACCGTTTTTTCAGGTTTTTTGAAG

GTGCCGGTGCAGTTGTTATGAATGGTCAAACCACCAAACCTGGAAAAAGGCG
ATATGTTTGTGTTCCGAGCTGGGTTCGGTGGTCACTGCAGGCAGAAACCCA
GTTTGACCTGTTTCGTTTTAGTGATGCTCCGATTATGGAAGCACTGAGCTTTAT
GCGTACCAAATTGAAAGGCCAGAAACATCATCATCATCATTAAGGGCCCC
CCCTCGAG

Sequence of *sgp1*:

ATTAAAGAGGAGAAAGGTACCATGAATACCGCACCGAATACCCAGAGCACCA
GCGCACGTGCAGCATATTATCGTCGTATTGATGCATTTGCACTGACACCGCTG
TGGGAAAGCCTGCATGCACTGGTTCCGAAAACCCCGACCACCGCAATTGAA
CCGGCACTGTGGCGTTATGCAGAA GTTCGTCCGCTGCTGGCAGAAAGCGGTG
AACTGATTAGCGCAATGGAA GCAGAACGTCGTGTTCTGATTCTGGAAAATCC
GGCACTGCGTGGTCA GAGCGCAATTACCCAGAGTCTGTATGCAGGTCTGCAG
CTGATTCTGCCTGGTGAAGTTGCACCGGCACATCGTCATAGCCAGAGCGCAC
TGCGTCTGGTCTGGAAGGTGAAACCGCATTACC GCAGTTGATGGTGAACG
TATTCCGATGACACCGGGTGATTTTATCATTACTCCGGCACATACCTGGCATGA
TCACGGTAATACCGGTAATGATGCAGTTGTTTGGCTGGATGGTCTGGATATTC
CGCTGGTTCGTTTTTTGAAGCAGGTTTTGCAGAAAAAGCCGAAGAAAGCG
TTCAGCGTGTTAGCCGTGCAGAA GGTGATAGCCTGGCACGTTATGGTAGCAA
TCTGGTTCGCTGGATAGCCGTCGTGATGCAGCACAGCCGAGCCGTCTGTGT
CATTATCCGTATAAACATAACCCGTGCAGCACTGGCAGATCTGGCACGTGGTGA
TGCA GATCCGCATTATGGTCATGCACTGCGTTTTAACAATCCGGAAACCGGTC
ATAGCCCGATGCCGACCATTGGCACCTTTATTCA GCTGCTGCCTGCCGGTTTT
GAAACCCGTACCCGTCGTAGCACCGATAGCACCAATTGTTTGTGCTGGAAG

GCGGTGGCACCGCACAGGTTGGTGAACGCACCTTTCA GTTTGCACAGAATGA
TATTTTTGTGATCCCGAGCTGGTCAGCCCTGCGTCTGCAGGCAGGTCCGGAA
ACAGTTCTGTTTAGCTATAGCGATCGTCCGGTTCAGCAGGCACTGGGTCTGTG
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Sequence of *mps1*:

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CACCGCATCTGTGGGAATTTGAAGTTGCAAAAAGAATTTCTGATGGAA GCAGG
CACCTGATTACCGCAAAGAAGCAGAACGTCGCGTACTGATTCTGGAAAAT
CCGGGTCTGAAAGGTCTGAGCCGTATTACCACCGTCTGTATGCAGGTCTGC
AGCTGATTCTGCCTGGTGAAGTGGCACCGACCCATCGTCATAGCCA GAGCGC
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CGTACCACCATGCAGGTTGGT GATTTTGTTATTACCCCTCCGTGGGCATGGCA
TGATCACGTTAATGATAGCGATAAACCGATGATTTGGATGGATGGTCTGGATC
TGCCGATGGTTACCCTGTTTGATAACGCTTTGCCGAAGGTTATGGTGAA GAT
ATTCAAGAAATTACCCGTCCGAATGGTGATAGCCTGGCACGTTATGGTGCAA
TATGCTGCCGGTTGATTTTAAACAGAAAGGCCTGAGCAGCCCGATTTTAACT
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ATGGCAGCAATGCCGACCATTAGCAGCTTTATTCAGCTGCTGCCGAAAGAATT
CCGTACCCAGACCTATCGTAGCACC GATGCAACCGTTTTTA GCGTTATTGAAG
GTCAGGGTAAAACCCGCATTGGTGATAAAGTGTTTTTCTGGAAAGCGAAAGA

TACCTTTGTTGTTCCGAGTTGGTATCCGGTTGAACATGAA GCAAGCA GTGATG
CAGTTCTGTTTAGCTATAGCGATCGTGTTGCACAGCAGAAACTGGGTTTTTGG
CGTGAAAGCCGTAATCATCATCATCATCATCATTAAGGGCCCCCCCTCGAG

Sequence of *mps2*:

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ATCCGGCACTGAAACAGCTGTATACCGATTTTGAA GCA GAACATCTGAATCC
GCTGTGGACACAGCTGGGTGATCTGATGCCGATGACCCCGACCAGCCGTGCA
GTTCCGTTTGTGGAAATGGTCAACCCTGTATCCGCTGGCACAGCGTGCCG
GTGATCTGGTCCGGTGGTTCGTGGTGGTGAACGTCGTGCAATTGCCCTGGC
AAATCCGGGTCTGGGTGGTGTTCGTATGTTACCCCGACCCTGTGGGCAGCA
ATTCAGTATCTGGGTCCGAAAGAA GTTGCACCGGAACATCGTCATGCACAGA
ATGCATTTCTTTTTGTTGTTGAA GGTGAAGGTGTTTGGACCGTTGTTAATGGT
GATCCGGTTGCAATGCGTCGTGGTGAATTTCTGCTGACACCGGGTTGGCATT
TCATGGTCATCATAATGAAACCGATCAGCCGATGGCATGGATTGATGGTCTGG
ATATCCCGTTTGTTCATTATACCGATAACCGGCTTTTTTTGAATTTGGCAGCGAAA
ATGTTACCGATGATAGCACACCGGATGTTAGCCGTAGCGAACGTCTGTGGGC
ACATCCTGGTCTGCGTCCGCTGGTTGGCCTGGATGCAAAAACCAGCAGCCCG
ATTGCAGCATATCGTTGGGAACACACCGATGCAGCACTGCGTGAACAGCTGG
CACTGGAAGATGAA GGTATGCA GCAACCACCGAACCGGGTCATGCAGCAG
TTCGTTATACCAATCCGACCACAGGTGGTGGTGGTATGTTATGCCGACCATTCTGCA
GAATTCATCGTCTGCGTGC GGGTGCACATAACCGTCCGCGTCGTGATGTTGG
TAGCACCGTTTATCAGGTTTTTTGAAGGTAGCGGTCGTTTTGTGCTGGGTGGTC
AGACCCGTACCGTGGGTAAAGGTGATATGATTGTTGTTCCGAGCTGGACCGA

ATGGTCAATTGAA GCCGATACCGAATTTGACCTGTTTGCATTTA GTGATGCAC
CGATTGTTGAACGTCTGCATTTTCACCGTACCTATATTAGCGAA GGTGCACAT
CATCATCATCATCATTAAGGGCCCCCCCCCTCGAG

Sequence of *mps3*:

ATTAAAGAGGAGAAAGGTACCATGAACAACAACGATAATATTGGCACCGCAC
GTGCAGATTTTTATCAGCGTATTGATAGCCAGGCACTGTTTCCGCTGTGGGAA
CAGCTGCATAATCTGGTTCGCCTCAGCCGACCACCACCTGTGTTCCGGCAC
TGTGGCGTTTTAGCGAAGTTCGTCCGTATCTGATGGAAGCAGGTCAGCTGATT
AGTGCCGAAGAAGCAGTTCGTCGCGTTC TGGTTCTGGAAAATCCGGGTATTC
GTGGTCAGGCAAGCATTACCCCGAGCCTGTATGCAGGTCTGCAGCTGATTCT
GCCTGGTGA AATTGCACCGAGCCATCGTC ATAGCCA GAGCGCACTGCGTTTT
GTTGTTGAA GGTAAA GGTGCATATAACCGCAGTTGATGGTGAACGTACCACCA
TGCATCCGGGTGATTTCAATTATACCCCGTCATGGACCTGGCATGATCACGGTA
ATCCGAGCGAAACCGAA GGTGGTGAACCGGTTATTTGGCTGGATGGTCTGGA
TATCCGACCGTTCGTTTTTATGGTGCAGGTTTTGCAGAAAATTATCCGCAGG
CAGTTCA GCCGGTTTCACGTCCGGATGGTAATAGCCTGGCACGTTATGGTGCG
AATATGCTGCCGCTGCGTCATGAA GTTCA GGGTGCAACCA GCCCGATTTTTAG
CTATCCGTATGCACGTACCCGTGAAGCACTGGCAACCCTGGAACAGCAGGGT
GAAGTTGATGCATGGGATGGTGTAAACTGCGTTATGTTAATCCGGCAACCGG
TGGTTGGGCAATGCCGACCATTGGTACATGCATGCAGCTGCTGCCTCGTGGTT
TTGCGGGTAAAACCGCACGTTGTACCGATGCAACCGTTTATA GCGTTGTGGA
AGGTCGTGGTCGTGCAATTATTGGTGGTCAGACCTTTCATTTTGAACCGAAA
GATACCTTTGTTGTTCCGAGCTGGGTGACCCTGAGTCTGCAGGCA GATGATG

CATGTGTTCTGTTTAGCTATAGCGATCGTCCGGTTCAA GAAGCCCTGGGTCTG
CTGCGTGAAGCCCGTGAACATCATCATCATCATATTAAGGGCCCCCCTCGA
G

Sequence of *aroF^{fbr}*:

GGATCCATTAAGAGGAGAAAGGTACCATGCAAAAAGACGCGCTGAATAAC
GTACATATTACCGACGAACAAGGTTTTAATGACTCCGGAACAACCTGAAAGGCCG
CTTTTCCATTGAGCCTGCAACAAGAAGCCAGATTGCTGACTCGCGTAAAAG
CATTCAGATATTATCGCCGGGCGCGATCCTCGTCTGCTGGTAGTATGTGGTCC
TTGTTCCATTCATGATCCGGAAACTGCTCTGGAATATGCTCGTCGATTTAAAG
CCCTTGCCGCA GAGGTCA GCGATA GCCTCTATCTGGTAATGCGCGTCTATTTT
GAAAACCCCGTACCACTGTCGGCTGGAAAGGGTTAATTAACGATCCCCATA
TGGATGGCTCTTTTGATGTAGAA GCCGGGCTGCA GATCGCGCGTAAATTGCT
GCTTGAGCTGGTGAATATGGGACTGCCACTGGCGACGGAA GCGTTAGATctgA
ATAGCCCGCAATACCTGGGC GATCTGTTTAGCTGGTCA GCAATTGGTGCTCGT
ACAACGGAATCGCAAACCTACCGTGAAATGGCCTCCGGGCTTTCCATGCCGG
TTGGTTTTAAAACGGCACCGACGGCAGTCTGGCAACAGCAATTAACGCTAT
GCGCGCCGCCGCCAGCCGCACCGTTTTGTTGGCATTAAACAGGCA GGGCAG
GTTGCGTTGCTACAAACTCAGGGGAATCCGGACGGCCATGTGATCCTGCGCG
GTGGTAAAGCGCCGAACTATAGCCCTGCGGATGTTGCGCAATGTGAAAAAGA
GATGGAACAGGC GGGACTGCGCCC GTCTCTGATGGTAGATTGCAGCCACGGT
AATTCCAATAAAGATTATCGCCGTCA GCCTGCGGTGGCAGAATCCGTGGTTGC
TCAAATCAAAGATGGCAATCGCTCAATTATTGGTCTGATGATCGAAAGTAATA
TCCACGAGGGCAATCA GTCTCCGAGCAACC GCGCA GTGAAATGAAATACG

GTGTATCCGTAACCGATGCCTGCATTAGCTGGGAAATGACCGATGCCTTGCTG
CGTGAAATTCATCAGGATCTGAACGGGCA GCTGACGGCTCGCGTGGCTTAAT
CTAGAGGCATCAAATAAAACAAGCTT

Sequence of *aroG^{fbr}*:

GGATCCATTAAGAGGAGAAAGGTACCATGAATTATCAGAACGACGATTTAC
GCATCAAAGAAATCAAAGAGTTACTTCCTCCTGTCGCATTGCTGGAAAAATT
CCCCGCTACTGAAAATGCCGCGAATACGGTTGCCCATGCCGAAAAGCGATC
CATAAGATCCTGAAAGGTAATGATGATCGCCTGTTGGTTGTGATTGGCCCATG
CTCAATTCATGATCCTGTCGCGGCAAAGAGTATGCCACTCGCTTGCTGGCGC
TGCGTGAAGAGCTGAAAGATGAGCTGGAAATCGTAATGC GCGTCTATTTTGA
AAAGCCGCGTACCACGGTGGGCTGGAAAGGGCTGATTAACGATCCGCATATG
GATAATAGCTTCCAGATCAACGACGGTCTGCGTATAGCCCGTAAATTGCTGCT
TGATATTAACGACAGCGGTCTGCCAGCGGCAGGTGAGTTTCTC_{aac}ATGATCA
CCCCACAATATCTCGCTGACCTGATGAGCTGGGGCGCAATTGGCGCACGTAC
CACCGAATCGCAGGTGCACCGCGAACTGGCATCAGGGCTTTCTTGTCCGGTC
GGCTTCAAAAATGGCACCGACGGTACGATTAAA GTGGCTATCGATGCCATTAA
TGCCGCCGGTGCGCCGCACTGCTTCTGTCCGTAACGAAATGGGGGCATTCG
GCGATTGTGAATACCAGCGGTAACGGCGATTGCCATATCATTCTGCGCGGCGG
TAAAGAGCCTAACTACAGCGCGAAGCACGTTGCTGAA GTGAAAGAAGGGCT
GAACAAAGCAGGCCTGCCAGCACAGGTGATGATCGATTTCAGCCATGCTAAC
TCGTCCAAACAATTCAAAAAGCAGATGGATGTTTGTGCTGACGTTTGCCAGC
AGATTGCCGGTGGCGAAAAGGCCATTATTGGCGTGATGGTGGAAA GCCATCT
GGTGGAAAGGCAATCAGAGCCTCGAGAGCGGGGAGCCGCTGGCCTACGGTAA

GAGCATCACCGATGCCTGCATCGGCTGGGAA GATACCGATGCTCTGTTACGTC
AACTGGCGAATGCAGTAAAAGCGCGTCGCGGGTAATTCTAGAGGCATCAAATA
AAACAAGCTT

Underline: Initiation and termination codons

*The codons encoding amino acid changes with respect to the original sequence are indicated as small letters.

Supplementary references

1. Noda, S., Shirai, T., Oyama, S. & Kondo, A. Metabolic design of a platform *Escherichia coli* strain producing various chorismate derivatives. *Metab. Eng.* **33**, 119–129 (2016).