Supplementary information

Full use of lignocellulosic biomass in efficient synthesis of L-tyrosine and its analogues by engineering microbial consortia

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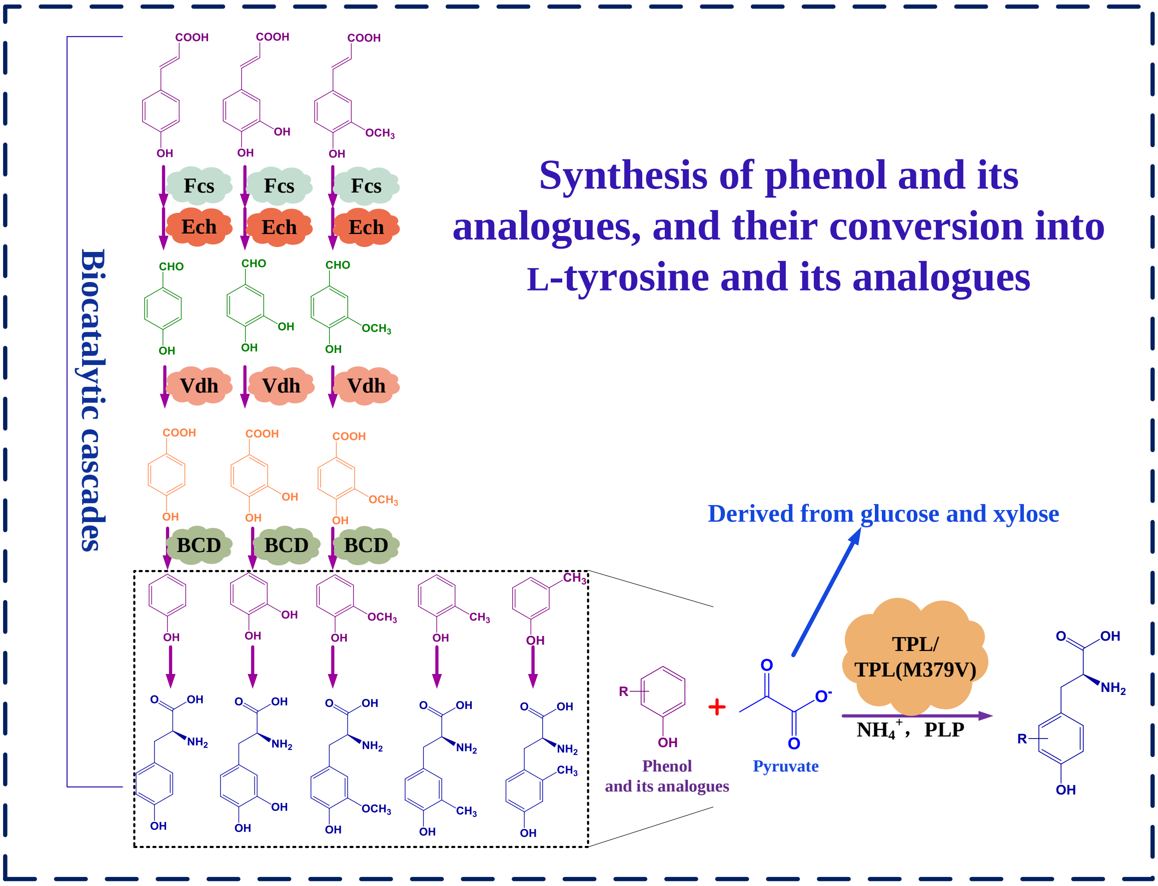

Fig. S1 Biocatalytic cascades towards the synthesis of L-tyrosine derivatives from lignin-derived phenolics and pyruvate produced from glucose-xylose mixtures.

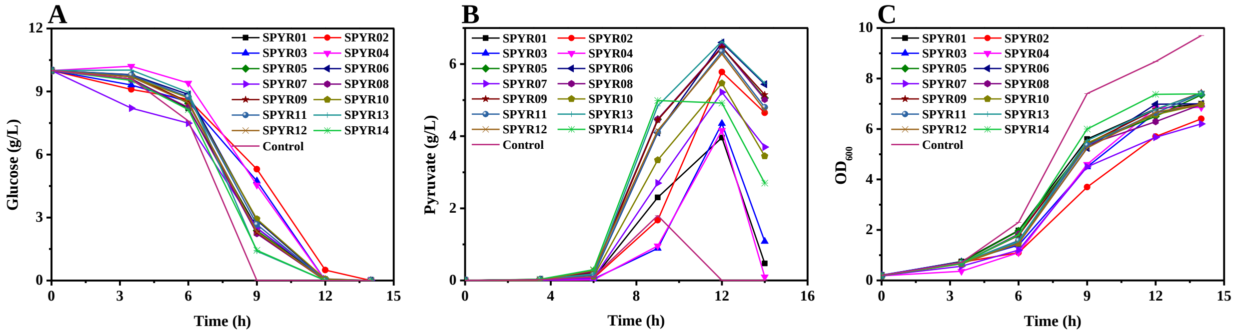

Fig. S2 Optimization of pyruvate synthesis within different strains containing different sgRNAs. A) Glucose consumption by different strains. B) Pyruvate synthesis by different strains. C) Cell growth of different strains. The colors used here are unrelated to those used elsewhere. The fermentations were started using 10 g L^{-1} glucose. When cell cultures reached an OD_{600} of 0.6, 0.35 mg L^{-1} anhydrotetracycline (aTc) was added for dCas9 expression except for the control experiments. The fermentation were finished after 14 h.

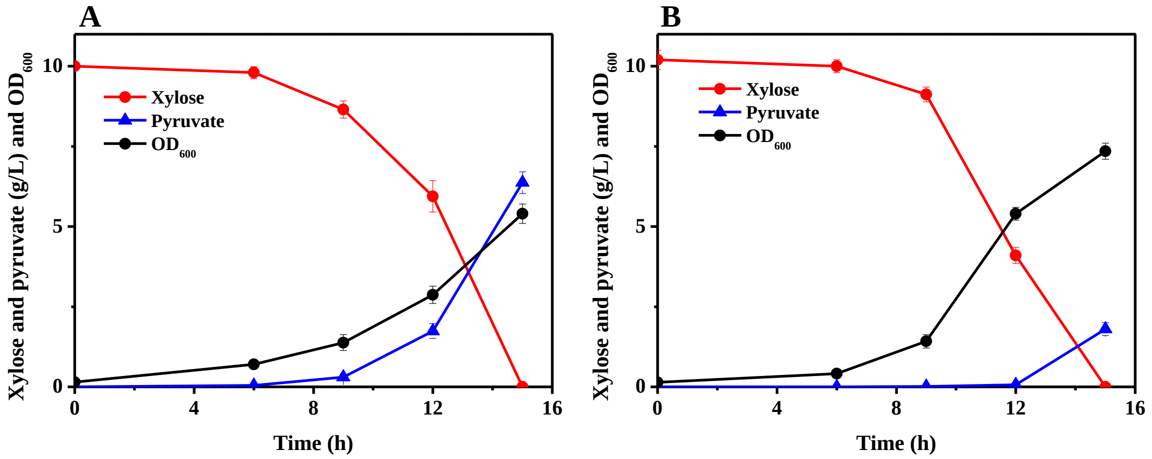

Fig. S3 Pyruvate synthesis using xylose-selective strains. A) Pyruvate synthesis using strain SPYR15 carrying a CRISPRi system. When cell cultures reached an OD_{600} of 0.6, 0.35 mg L^{-1} aTc was added for dCas9 expression. B) Control experiment using strain MG74A3.

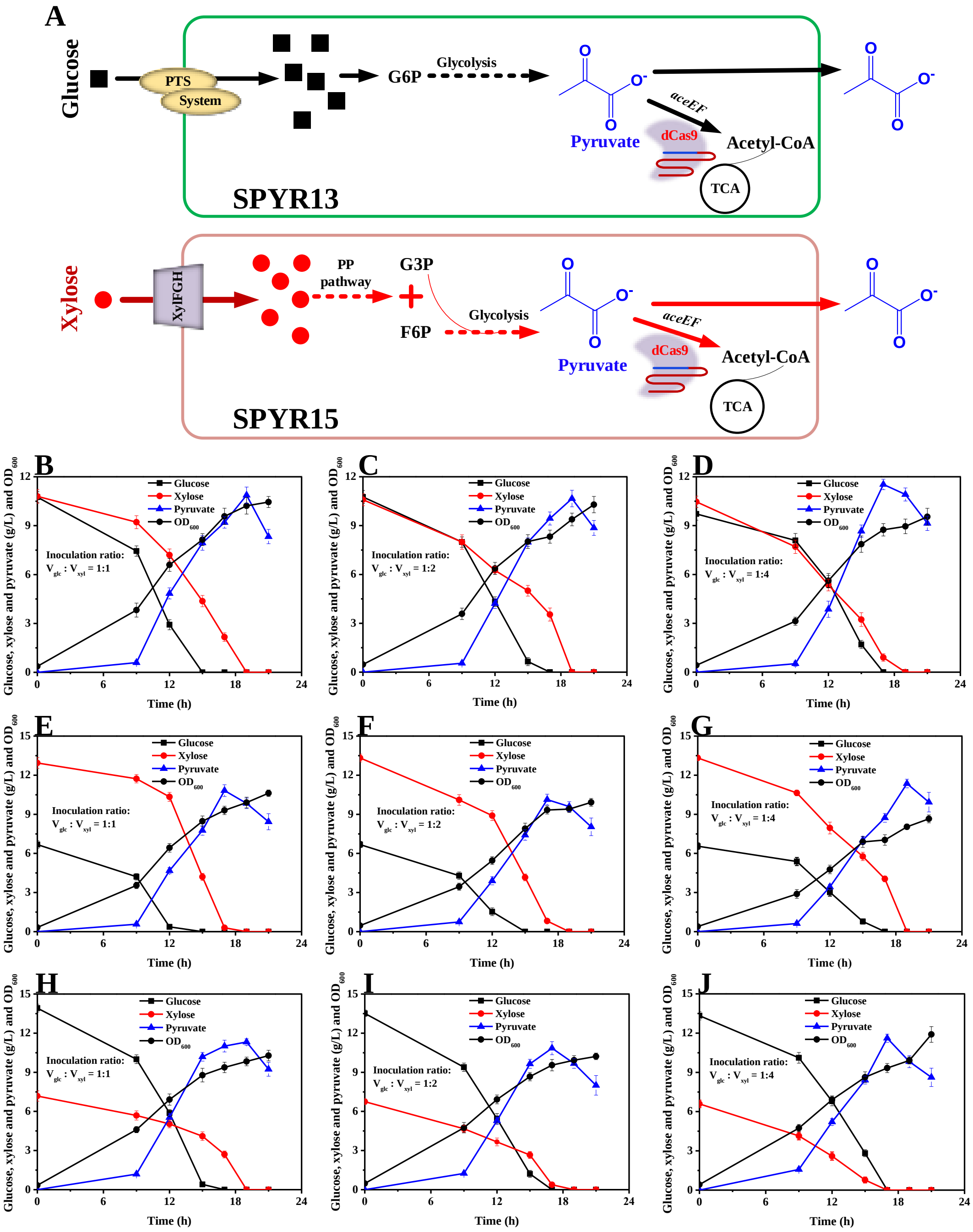

Fig. S4 Optimization of pyruvate synthesis using the SPYR13-SPYR15 consortium with both of different inoculation ratios of SPYR13 to SPYR15 and different concentrations of glucose and xylose. A) Construction of SPYR13-SPYR15 consortium for pyruvate synthesis. The fermentations were started using 10 g L^{-1} glucose and 10 g L^{-1} xylose with an inoculation ratio of 1:1 (SPYR13:SPYR15) (B), 1:2 (SPYR13:SPYR15) (C) and 1:4 (SPYR13:SPYR15) (D). The fermentations were started using 6.5 g L^{-1} glucose and 13.5 g L^{-1} xylose with an inoculation ratio of 1:1 (SPYR13:SPYR15) (E), 1:2 (SPYR13:SPYR15) (F) and 1:4 (SPYR13:SPYR15) (G). The fermentations were started using 13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose with an inoculation ratio of 1:1 (SPYR13:SPYR15) (H), 1:2 (SPYR13:SPYR15) (I) and 1:4 (SPYR13:SPYR15) (J). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

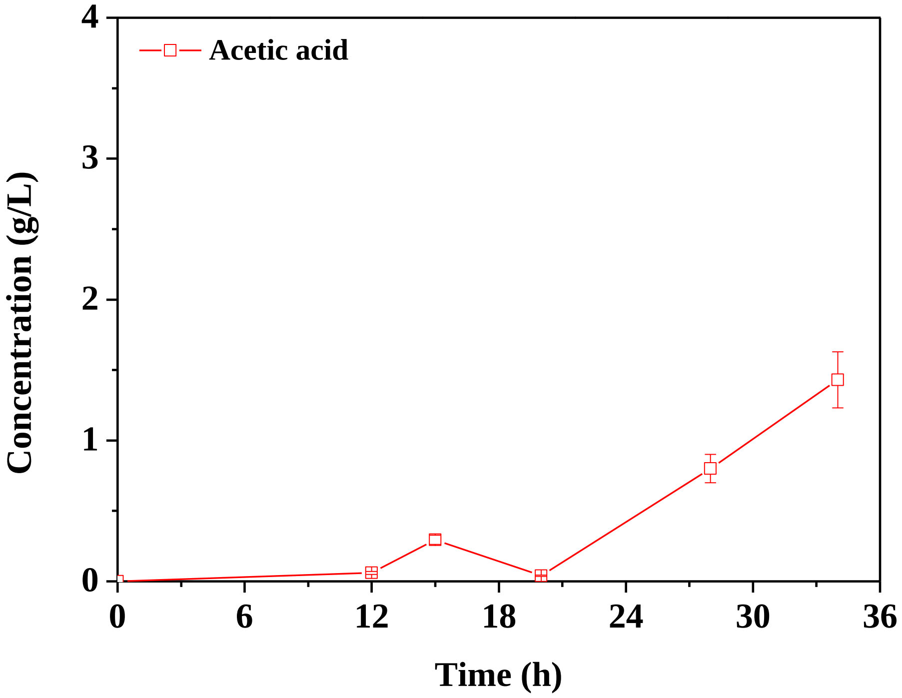

Fig. S5 The time course for the accumulation of acetic acid during the process of producing pyruvate with the SPYR13–SPYR15 consortium.

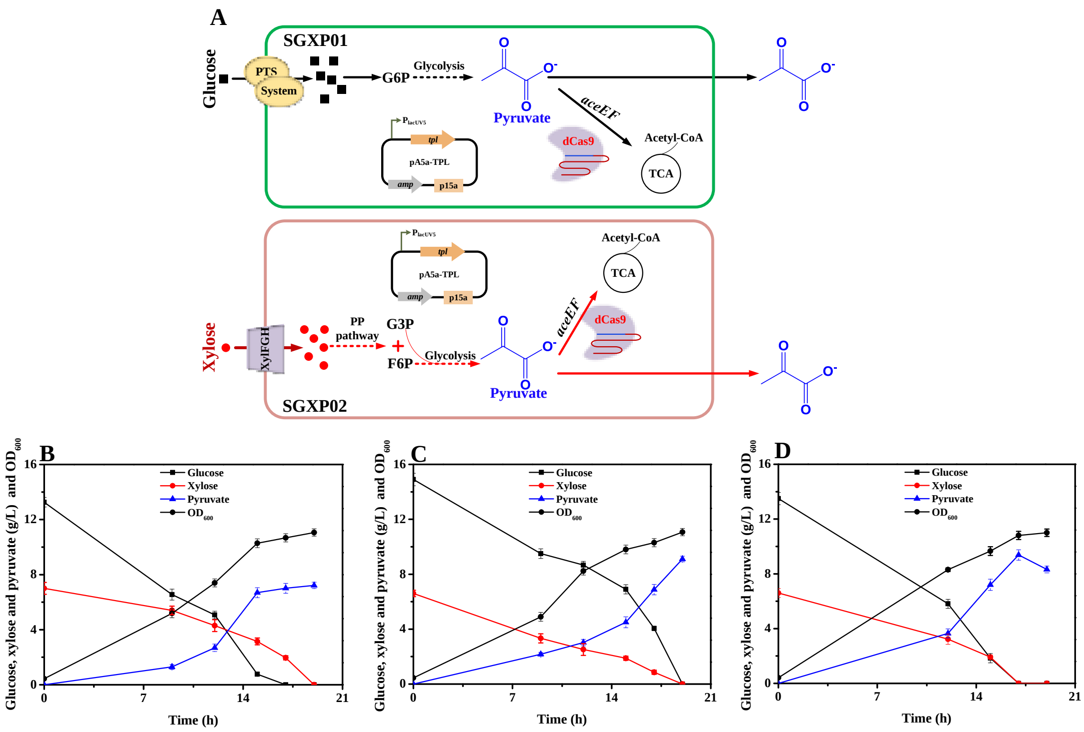

Fig. S6 Optimization of pyruvate synthesis using the SGXP01-SGXP02 consortium with different inoculation ratios and 20 g L^{-1} glucose-xylose mixtures (13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose). A) Construction of SGXP01-SGXP02 consortium for pyruvate synthesis. The fermentations were started using an inoculation ratio of 1:1 (SGXP01:SGXP02) (B), 1:2 (SGXP01:SGXP02) (C) and 1:4 (SGXP01:SGXP02) (D). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

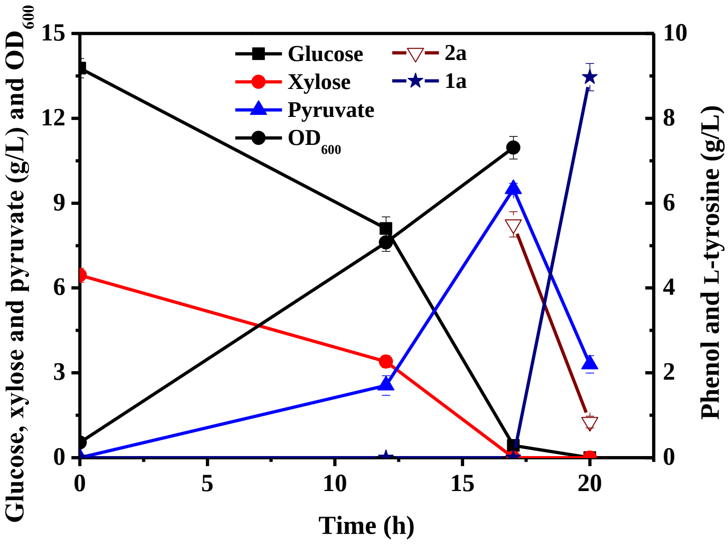

Fig. S7 The time course for the synthesis of **1a** from 5.5 g L^{-1} **2a** with the GXP system. The fermentations were started using 13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose. After 17 h of fermentation, 5.5 g L^{-1} **2a** was added with 20 g L^{-1} NH_4Cl and 0.5 mM PLP to begin the synthesis of **1a**.

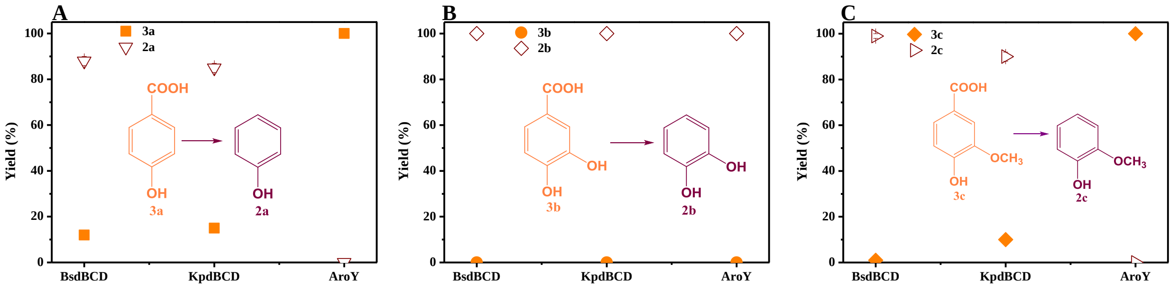

Fig. S8 Comparison of different sources of decarboxylases to catalyze the decarboxylation of lignin-derived hydroxybenzoic acids to phenol and its analogues. A) Decarboxylation of *p*-hydroxybenzoic acid (**3a**) to produce phenol (**2a**). B) Decarboxylation of protocatechuic acid (**3b**) to produce catechol (**2b**). C) Decarboxylation of vanillic acid (**3c**) to produce guaiacol (**2c**). The genes (*BsdBCD*, *KpdBCD* and *aroY*) were cloned into plasmid pA5a and then were transformed into *E. coli* MG1655 to construct three biocatalysts. The reactions were performed in 10 mL KPi buffer with an OD_{600} of 10 at 37 ℃ and 220 rpm for 1 h.

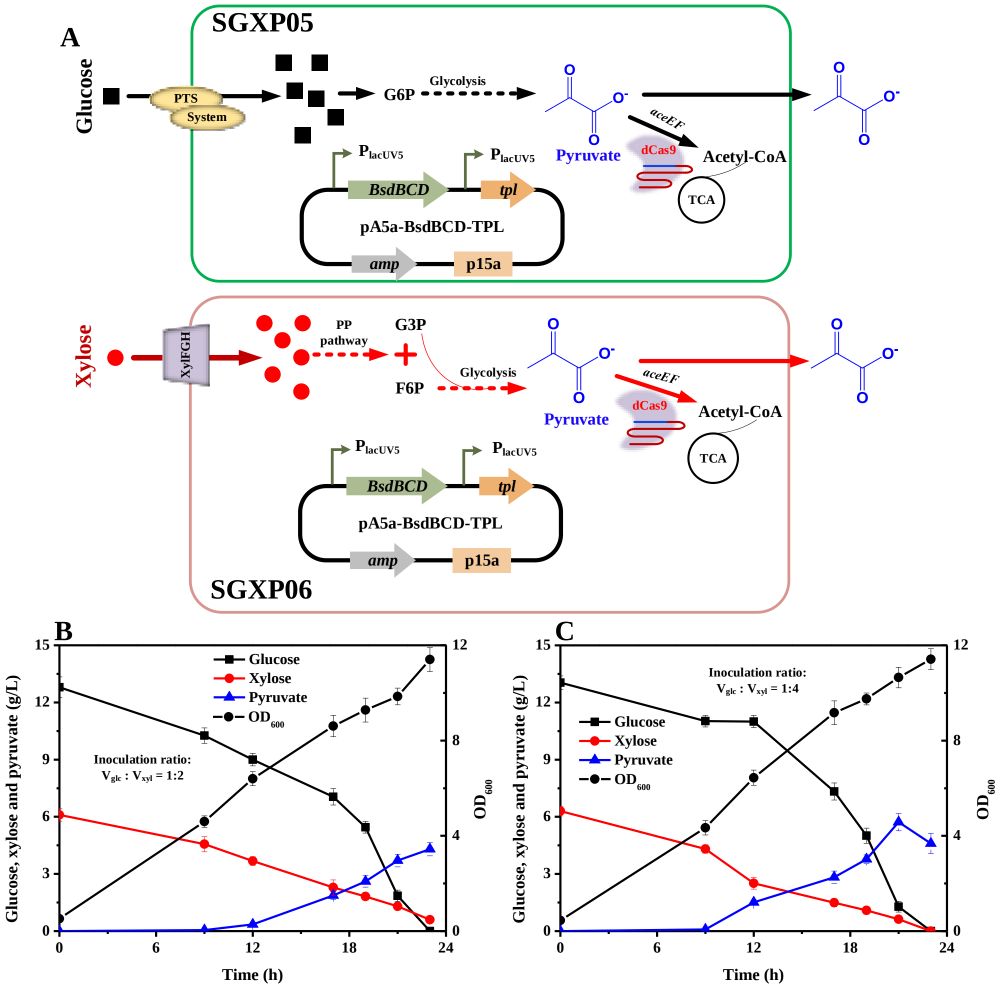

Fig. S9 Optimization of pyruvate synthesis using the SGXP05-SGXP06 consortium with different inoculation ratios and 20 g L^{-1} glucose-xylose mixtures (13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose). A) Construction of SGXP05-SGXP06 consortium for pyruvate synthesis. The fermentations were started using an inoculation ratio of 1:2 (SGXP05:SGXP06) (B) and 1:4 (SGXP05:SGXP06) (C). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

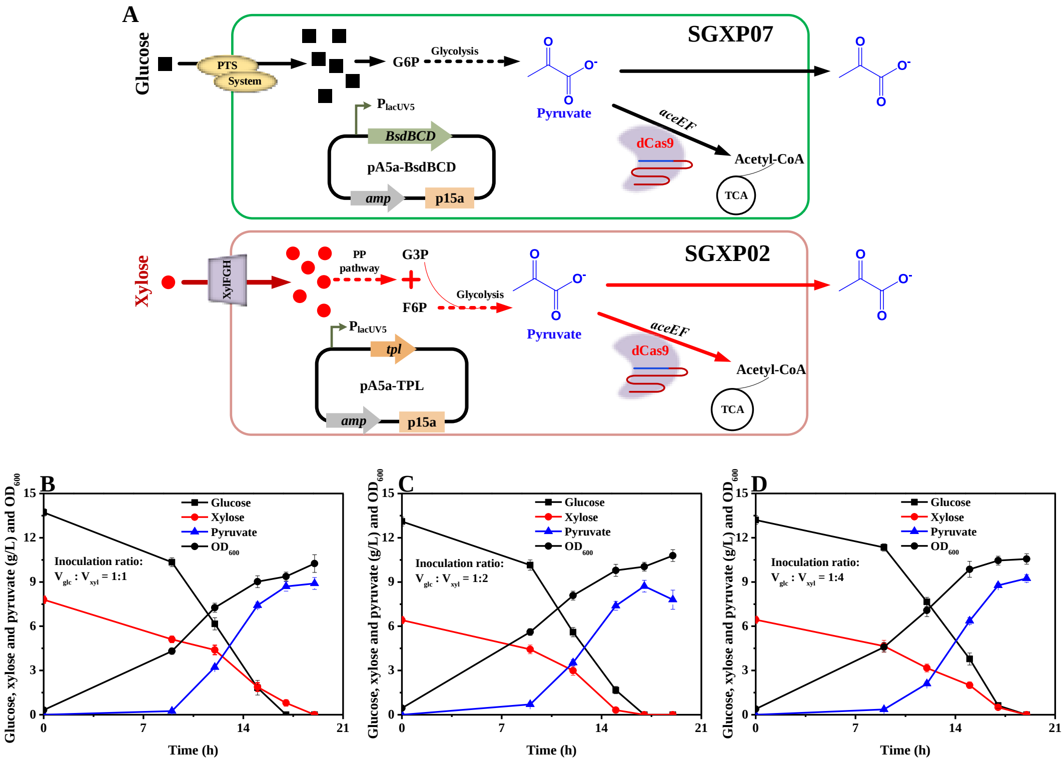

Fig. S10 Optimization of pyruvate synthesis using the SGXP07-SGXP02 consortium with different inoculation ratios and 20 g L^{-1} glucose-xylose mixtures (13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose). A) Construction of SGXP07-SGXP02 consortium for pyruvate synthesis. The fermentations were started using an inoculation ratio of 1:1 (SGXP07:SGXP02) (B), 1:2 (SGXP07:SGXP02) (C) and 1:4 (SGXP07:SGXP02) (D). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

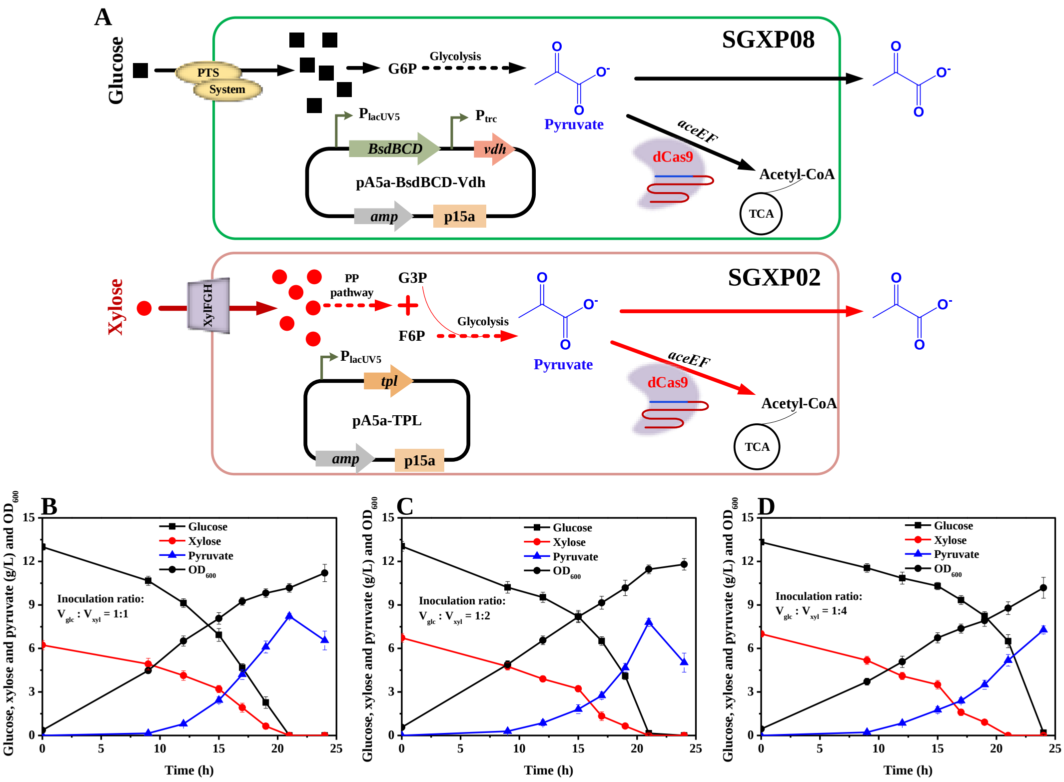

Fig. S11 Optimization of pyruvate synthesis using the SGXP08-SGXP02 consortium with different inoculation ratios and 20 g L^{-1} glucose-xylose mixtures (13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose). A) Construction of SGXP08-SGXP02 consortium for pyruvate synthesis. The fermentations were started using an inoculation ratio of 1:2 (SGXP08:SGXP02) (B), 1:2 (SGXP08:SGXP02) (C) and 1:4 (SGXP08:SGXP02) (D). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

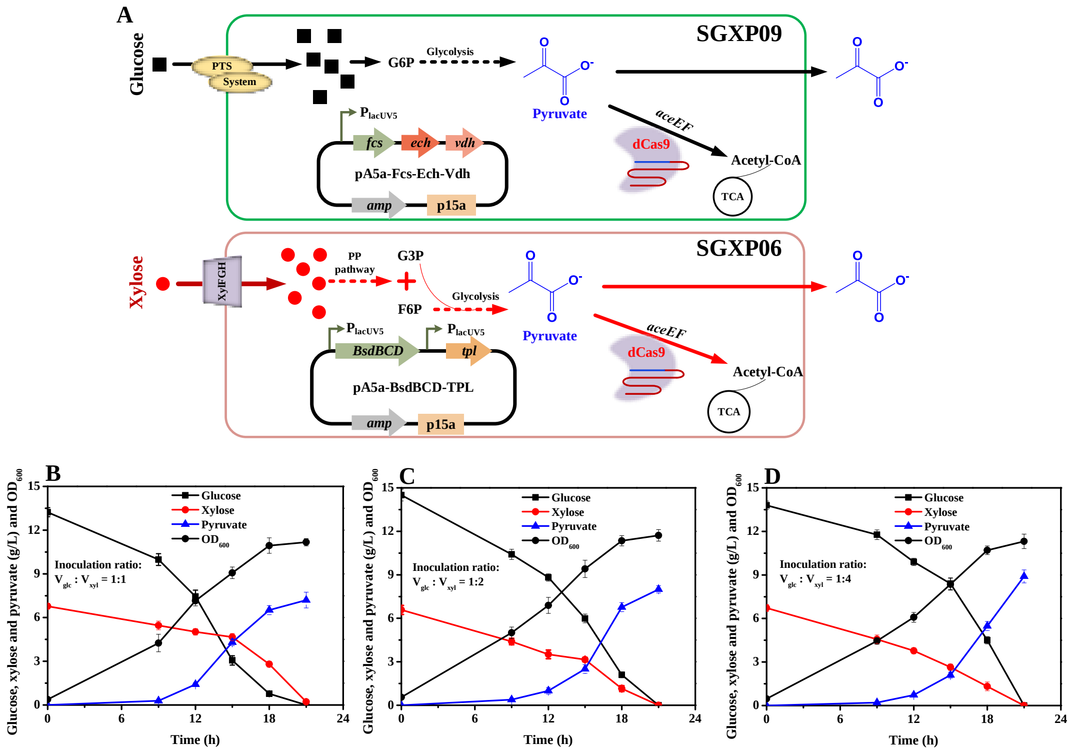

Fig. S12 Optimization of pyruvate synthesis using the SGXP09-SGXP06 consortium with different inoculation ratios and 20 g L^{-1} glucose-xylose mixtures (13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose). A) Construction of SGXP09-SGXP06 consortium for pyruvate synthesis. The fermentations were started using an inoculation ratio of 1:2 (SGXP09:SGXP06) (B), 1:2 (SGXP09:SGXP06) (C) and 1:4 (SGXP09:SGXP06) (D). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression.

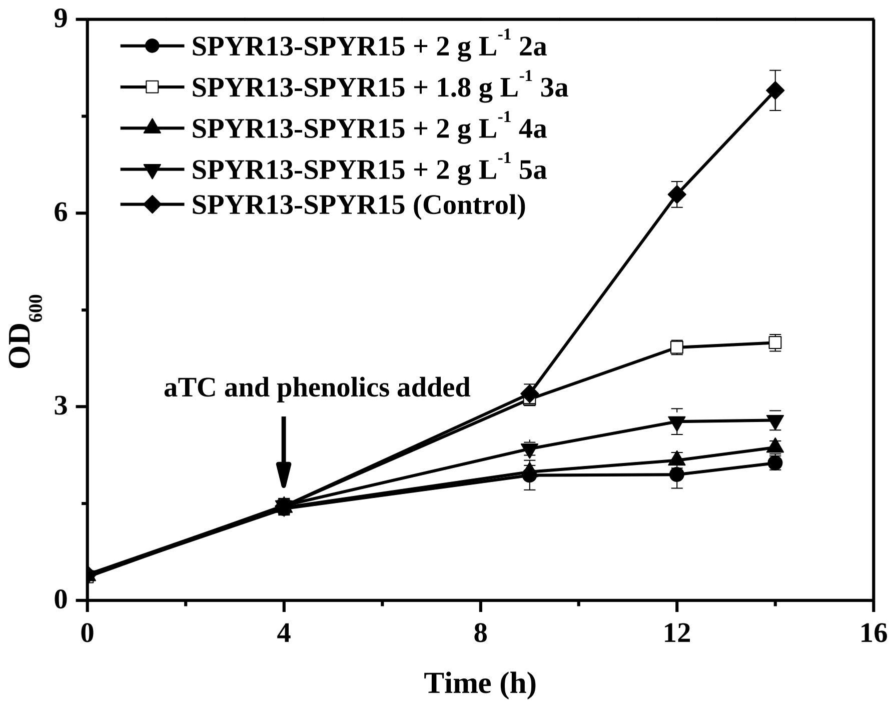

Fig. S13 The effects of the different phenolics on the growth of SPYR13-SPYR15 consortium. The fermentations were started using 13.5 g L^{-1} glucose and 6.5 g L^{-1} xylose with an inoculation ratio of 1:4 (SPYR13:SPYR15). When cell cultures reached an OD_{600} of 1.2, 0.35 mg L^{-1} aTc was added for dCas9 expression, and different concentrations of phenolics (2 g L^{-1} **2a**, 1.8 g L^{-1} **3a**, 2 g L^{-1} **4a** and 2 g L^{-1} **5a**) were added to test the growth of SPYR13-SPYR15 consortium.

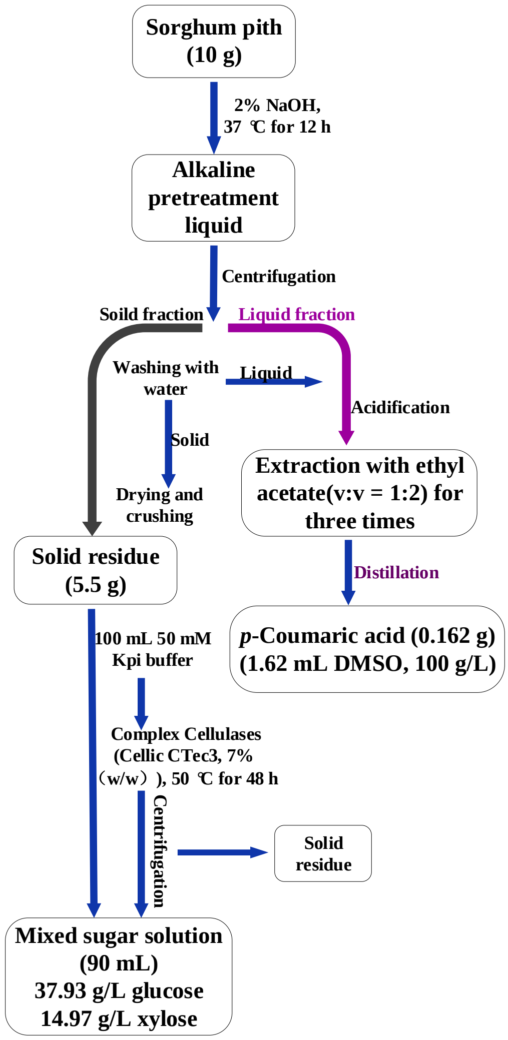

Fig. S14 The entire enzymatic-chemical process to obtain the glucose-xylose mixtures and *p*-coumaric acid (**5a**) from sorghum pith.

Table S1. Plasmids used in this study

Plasmids	Descriptions	Sources
pS2k	Tet promoter, pSC101 ori, Kan^R	BglBrick[1]
pA5a	LacUV5 promoter, p15A ori, Amp^R	BglBrick[1]
pB1c	Trc promoter, pBBR1 ori, Cm^R	BglBrick[1]
pCas	*repA101*(Ts) *kan* *Pcas-cas9* *ParaB-Red* *lacI*q *Ptrc*-sgRNA-*pMB1*	[2]
pTargetF	P_{J23119}, pMB1 ori, Sm^R	[2]
pS2k-dcas9	pS2k carrying *dcas9*	This study
pTargetF-*aceE*01	N20-1, P_{J23119}-sgRNA-*aceE*1, pMB1 ori, Sm^R	This study
pTargetF-*aceE*02	N20-2, P_{J23119}-sgRNA-*aceE*2, pMB1 ori, Sm^R	This study
pTargetF-*aceE*03	N20-3, P_{J23119}-sgRNA-*aceE*3, pMB1 ori, Sm^R	This study
pTargetF-*aceE*04	N20-4, P_{J23119}-sgRNA-*aceE*4, pMB1 ori, Sm^R	This study
pTargetF-*aceE*05	N20-5, P_{J23119}-sgRNA-*aceE*5, pMB1 ori, Sm^R	This study
pTargetF-*aceE*06	N20-6, P_{J23119}-sgRNA-*aceE*6, pMB1 ori, Sm^R	This study
pTargetF-*aceE*07	N20-7, P_{J23119}-sgRNA-*aceE*7, pMB1 ori, Sm^R	This study
pTargetF-*aceE*08	N20-8, P_{J23119}-sgRNA-*aceE*8, pMB1 ori, Sm^R	This study
pTargetF-*aceE*09	N20-9, P_{J23119}-sgRNA-*aceE*9, pMB1 ori, Sm^R	This study
pTargetF-*aceE*10	N20-10, P_{J23119}-sgRNA-*aceE*10, pMB1 ori, Sm^R	This study
pTargetF-*aceE*11	N20-11, P_{J23119}-sgRNA-*aceE*11, pMB1 ori, Sm^R	This study
pTargetF-*aceE*12	N20-12, P_{J23119}-sgRNA-*aceE*12, pMB1 ori, Sm^R	This study
pTargetF-*aceE*13	N20-13, P_{J23119}-sgRNA-*aceE*13, pMB1 ori, Sm^R	This study
pTargetF-*aceE*14	N20-14, P_{J23119}-sgRNA-*aceE*14, pMB1 ori, Sm^R	This study
pTargetT-*ldhA*	P_{J23119}-sgRNA-*ldhA*, pMB1 ori, Sm^R, donor DNAs	This study
pTargetT-*poxB*	P_{J23119}-sgRNA-*poxB*, pMB1 ori, Sm^R, donor DNAs	This study
pTargetT-*pflB*	P_{J23119}-sgRNA-*pflB*, pMB1 ori, Sm^R, donor DNAs	This study
pA5a-TPL	pA5a carrying *tpl*	This study
pA5a-TPL(M379V)	pA5a carrying *tpl(M379V)*	This study
pA5a-BsdBCD	pA5a carrying *BsdBCD*	This study
pA5a-BsdBCD-Vdh	pA5a carrying *BsdBCD* and *vdh*	This study
pA5a-BsdBCD-TPL	pA5a carrying *BsdBCD* and *tpl*	This study
pA5a-BsdBCD-TPL(M379V)	pA5a carrying *BsdBCD* and *tpl(M379V)*	This study
pA5a-Fcs-Ech-Vdh	pA5a carrying *fcs*, *ech* and *vdh*	This study
pA5a-KpdBCD	pA5a carrying *KpdBCD*	This study
pA5a-AroY	pA5a carrying *aroY*	This study

Table S2. Strains used in this study

Strains	Descriptions	Sources
E. coli DH5α	*F*$^{-}$, *φ80d lacZΔM15, Δ(lacZYA-argF) U169, recA1, endA1, hsdR17(rk*$^{-}$*, mk*$^{+}$*), phoA, supE44λ*$^{-}$*, thi*$^{-1}$*, gyrA96, relA1*	
E. coli MG1655	K-12; *F*$^{-}$ λ^{-} *rph-1*	
MG40B	*E. coli* MG1655, *ΔldhA*, *ΔpoxB*, *ΔpflB*	This study
MG74A3	MG40B, *ΔptsG*, *ΔmanZ*, *ΔgalP*, *Δglk*	This study
SPYR01	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*01	This study
SPYR02	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*02	This study
SPYR03	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*03	This study
SPYR04	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*04	This study
SPYR05	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*05	This study
SPYR06	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*06	This study
SPYRP7	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*07	This study
SPYR08	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*08	This study
SPYR09	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*09	This study
SPYR10	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*10	This study
SPYR11	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*11	This study
SPYR12	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*12	This study
SPYR13	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*13	This study
SPYR14	MG40B carrying pS2k-dcas9 and pTargetF-*aceE*14	This study
SPYR15	MG74A3 carrying pS2k-dcas9 and pTargetF-*aceE*13	This study
SGXP01	SPYR13 carrying pA5a-TPL	This study
SGXP02	SPYR15 carrying pA5a-TPL	This study
SGXP03	SPYR13 carrying pA5a-TPL(M379V)	This study
SGXP04	SPYR15 carrying pA5a-TPL(M379V)	This study
SGXP05	SPYR13 carrying pA5a-BsdBCD-TPL	This study
SGXP06	SPYR15 carrying pA5a-BsdBCD-TPL	This study
SGXP07	SPYR13 carrying pA5a-BsdBCD	This study
SGXP08	SPYR13 carrying pA5a-BsdBCD-Vdh	This study
SGXP09	SPYR13 carrying pA5a-Fcs-Ech-Vdh	This study
SGXP10	SPYR15 carrying pA5a-BsdBCD-TPL(M379V)	This study

Table S3. N20 sequences (targeting sites) used for targeting *aceE*

N20	Sequences (5'-3')	PAM (NGG)
1	CGAAGCGCGTACTTTCGGTA	TGG
2	GGATGACCGATTCGATCGCC	TGG
3	CTTTCCGGCGAGAGTTCAAT	GGG
4	TCAACGTTATTAGATAGATA	AGG
5	TGCGGGCTTCAGCAAGCAGT	TGG
6	GTTGCTGATACCTGTGCCTG	CGG
7	AGCGGATAGCTGAACGAATA	CGG
8	AATGGTTGCGGAAGACTGGA	AGG
9	GGCGACCTGGTTTACTTCCA	GGG
10	CCAGACCCATAGATACGGTC	GGG
11	CCGGGCCGTCAAGACGCTGC	AGG
12	TCGATATCTGCATCAGACAC	CGG
13	TCTTCGGATCGTGACCACCA	CGG
14	GCGCCGAAGTCTTGCAGGCT	CGG

Table S4. Conversions (%) for the formation of tyrosine derivatives employing wild-type TPL from *Citrobacter freundii*.

Substrate	Conversion (%)
Guaiacol (**2c**)	n.c.
o-Cresol (**2d**)	n.c.
m-cresol (**2e**)	45

Reaction conditions: 1 g L^{-1} phenol derivatives, 20 g L^{-1} NH_4Cl, 10 g L^{-1} pyruvate, and 0.5 mM PLP. n.c.=no conversion. The biocatalyst was constructed by transforming pA5a-TPL into strain MG40B. The reactions were performed in 10 mL KPi buffer with an OD_{600} of 15 at 37 ℃ and 220 rpm for 6 h.

Table S5. Chemicals used in this study

Chemicals	Sources
Catechol (**2b**)	Macklin
o-Cresol (**2d**)	Macklin
Vanillin (**4c**)	Macklin
Ferulic acid **(5c**)	Macklin
m-Cresol (**2e**)	Aladdin
Caffeic acid (**5b**)	Aladdin
Protocatechuic acid (**3b**)	Shanghai yuanye Bio-Technology
Vanillic acid (**3c**)	Shanghai yuanye Bio-Technology
Protocatechualdehyde (**4b**)	Shanghai yuanye Bio-Technology
L-DOPA (**1b**)	Adamas
p-Coumaric acid (**5a**)	Adamas
p-Hydroxybenzoic acid (**3a**)	Shanghai Dibai
p-Hydroxybenzaldehyde (**4a**)	J&K Scientific
Phenol (**2a**)	Shanghai Titan Scientific
Guaiacol (**2c**)	RHAWN
L-Tyrosine (**1a**)	Sangon Biotech
3-OCH_3-L-tyrosine (**1c**)	Amatek Chemical
3-CH_3-L-tyrosine (**1d**)	Asta Tech

Table S6. DNA sequences used in this study

Gene name	DNA sequences
fcs	ATGAATAACGAAGCCCGCTCAGGGTCGACCGACCCTGGCCAACGTCCGCGCTA CCGCCAGGTGGCCATCGGGCATCCCCAGGTGCAGGTCAGTCACGTCGACGACG TGCTGCGCATGCAACCTGTCGAGCCACTGGCGCCGCTGCCGGCGCGCCTGCTC GAGCGCCTGGTGCATTGGGCCCAGGTGCGCCCGGACACCACTTTCATCGCGGC ACGCCAGGCAGACGGTGCCTGGCGTTCGATCAGCTACGTGCAGATGCTCGCCG ATGTGCGCACCATCGCCGCCAACTTGCTAGGACTGGGCCTCAGTGCCGAGCGC CCGCTGGCGCTGCTTTCCGGCAACGACATCGAACACCTGCAAATCGCCCTCGG CGCCATGTATGCCGGTATTGCCTATTGCCCGGTGTCGCCGGCCTACGCGCTGTTG TCGCAAGACTTCGCCAAGTTGCGCCATGTCTGCGAGGTGCTCACCCCCGGAGT GGTCTTCGTCAGCGACAGCCAGCCGTTCCAGCGCGCCTTCGAGGCGGTGCTGG ACGATTCGGTCGGCGTGATCAGCGTGCGTGGCCAGGTCGCAGGTCGCCCCCAT ATAAGCTTCGACAGCCTGTTGCAACCGGGTGACCTGGCGGCGGCCGATGCGGC TTTCGCCGCCACCGGGCCGGACACCATCGCCAAATTCCTCTTCACCTCGGGCTC GACCAAGCTGCCCAAGGCGGTGATCACCACCCAGCGCATGCTGTGCGCCAATC AGCAGATGCTTCTGCAGACTTTTCCGACGTTCGCCGAGGAGCCGCCGGTGCTG GTGGACTGGCTGCCGTGGAACCACACGTTCGGCGGTAGCCACAACCTCGGCAT CGTGCTTTACAACGGGGGCAGTTTCTACCTGGACGCCGGCAAGCCGACCCCGC AAGGCTTCGCCGAAACCTTGCGCAATCTGCGCGAGATTTCCCCCACGGCCTAC CTCACCGTACCCAAGGGCTGGGAGGAACTGGTCAAGGCACTGGAGCAGGACC CCGCGCTACGCGAGGTGTTCTTTGCCCGCATCAAGCTGTTCTTCTTTGCCGCCG CAGGCCTGTCGCAAAGCGTCTGGGACCGGCTGGACCGCATTGCCGAGCAACA CTGTGGCGAACGCATCCGCATGATGGCCGGCCTTGGCATGACCGAAGCCTCGC CATCGTGCACCTTCACCACCGGGCCTTTGTCGATGGCCGGCTATGTCGGGCTGC CGGCACCTGGCTGCGAAGTGAAGCTGGTGCCGGTGGGCGACAAGCTCGAGGC GCGCTTCCGTGGCCCGCATATCATGCCGGGCTACTGGCGCTCGCCGCAGCAGAC CGCCGAGGCGTTCGACGAGGAGGGCTTCTACTGTTCGGGCGACGCGTTGAAGC TGGCCGATGCCAGGCAGCCCGAGCTTGGCCTGATGTTCGATGGCCGTATCGCTG AGGACTTCAAACTTTCGTCCGGGGTATTCGTCAGTGTCGGGCCGCTGCGCAAC CGCGCAGTGCTGGAGGGCTCGCCTTACGTACAGGACATCGTGGTCACCGCGCC GGACCGTGAATGCCTGGGCCTGCTGGTGTTCCCGCGTCTGCCCGAGTGTCGGC GCCTGGCCGGGCTGGCAGAGGATGCCAGCGATGCGCGGGTGCTGGCCAACGA CACCGTGCGCAGTTGGTTCGCTGACTGGCTGGAGCGCTTGAACCGCGATGCCC AAGGCAACGCCAGCCGTATCGAATGGCTGTCGCTGCTGGCCGAGCCGCCGTCG ATCGACGCCGGTGAAATCACCGACAAGGGCTCGATCAATCAGCGCGCCGTGCT GCAGCGGCGCGCCGCTCAGGTCGAGGCGCTGTACCGTGGCGAAGACCCCGAC GCATTGCACGCCAAGGTGCGGCCTTGA
ech	ATGAGCAAATACGAAGGCCGCTGGACCACCGTGAAGGTCGAACTGGAAGCGG GCATCGCCTGGGTGACCCTCAATCGCCCGGAAAAACGCAATGCCATGAGCCCC ACCCTGAACCGGGAAATGGTCGACGTGCTGGAAACCCTTGAGCAGGACGCTG ACGCTGGCGTGCTGGTATTGACCGGTGCCGGCGAGTCCTGGACCGCCGGCATG GACCTGAAGGAGTACTTCCGCGAGGTGGACGCCGGCCCGGAAATCCTCCAGG

	AAAAGATTCGTCGCGAAGCCTCGCAATGGCAATGGAAGTTGCTGCGTCTGTAT GCCAAACCGACCATCGCCATGGTCAACGGCTGGTGCTTCGGCGGCGGCTTCAG CCCACTGGTGGCATGCGACCTGGCGATCTGCGCCAACGAAGCGACCTTCGGCC TGTCGGAAATCAACTGGGGCATCCCGCCTGGTAACCTGGTCAGCAAGGCCATG GCCGATACCGTTGGCCATCGTCAGTCGCTGTACTACATCATGACCGGCAAGACC TTCGATGGTCGCAAGGCTGCCGAGATGGGCCTGGTGAACGACAGTGTGCCGCT GGCCGAGCTGCGTGAAACCACCCGCGAGTTGGCGCTGAACCTGCTGGAAAAG AACCCGGTGGTGCTGCGTGCCGCGAAGAATGGCTTCAAGCGTTGCCGCGAGCT GACCTGGGAACAGAACGAGGACTACCTCTACGCCAAGCTCGACCAGTCGCGC CTGCTGGACACTACCGGCGGCCGCGAGCAGGGCATGAAGCAGTTCCTCGACGA CAAGAGCATCAAGCCAGGCCTGCAGGCCTACAAGCGCTGA
vdh	ATGTTGCAGGTGCCTTTGCTGATTGGCGGGCAGTCGCGCCCCGCCAGCGATGG ACGAACCTTCGAGCGCTGTAACCCGGTGACTGGCGAGGTGGTGTCGCAGGCTG CCGCCGCCACACTGGCCGATGCCGATGCCGCGGTGGCTGCTGCCAGCGCGGCG TTTCCGGCCTGGGCCGCCCTGGCACCGGGCGAGCGGCGCAGCCGCTTGCTGGC AGGCGCTGATCTGTTGCAGGCGAGGGCCGCCGAGTTCATCGCCGCCGCCGGTG AAACCGGGGCCATGGCCAACTGGTATGGCTTCAACGTGAAGTTGGCCGCCAAC ATGCTGCGCGAGGCTGCAGCCATGACCACGCAGATCACCGGTGAAGTGATCCC CTCGGACGTTCCCGGCAGCTTCGCAATGGCCCTGCGCGCGCCCTGCGGCGTGG TGTTGGGCATCGCACCGTGGAACGCCCCGGTGATACTGGCCACGCGTGCCATT GCCATGCCGCTGGCCTGCGGCAACACCGTGGTGCTCAAGGCCTCGGAGCTGAG CCCGGCGGTCCATCGGCTGATCGGCCAGGTGCTGCACGATGCAGGCATCGGCG ACGGCGTGGTCAATGTCATCAGCAATGCGCCGCAGGATGCCCCCGCCATCGTC GAGCGGCTGATCGCCAACCCTGCGGTACGCCGGGTCAACTTCACCGGTTCGAC GCACGTCGGGCGCATCGTCGGCGAACTGGCGGCCCGCCATCTCAAGCCGGCCC TGCTCGAACTGGGCGGCAAGGCACCTTTGCTGGTGCTCGACGATGCCGACCTG GACGCCACGGTCGAAGCGGCGGCCTTCGGTGCCTACTTCAACCAGGGGCAGAT CTGCATGTCCACCGAGCGCCTTGTGGTGGACAGCTGTATTGCCGACGCTTTCGT CGACAAGCTGGCGGTGAAGATCGCCGGGCTGCGTGCAGGTGATCCGCAAGCC AGCACCTCGGTGCTCGGCTCGCTGGTCAGCGCAGCGGCCGGCGAGCGCATCAA GGCACTGATCGACGATGCCGTGGCCAAGGGCGCGCGCCTGGTCAGCGGCGGC CAGCTGGAAGGCAGCATCCTGCAACCGACCTTGCTCGACAACGTCGATGCCAG CATGCGCCTGTACCGCGAGGAGTCCTTCGGCCCGGTGGCGGTGGTACTGCGCG CCGAAGGCGACGAAGCCTTGCTGCAGCTGGCCAACGACTCGGAGTTCGGTCTG TCATCGGCCATTTTCAGCCGCGACACCAGCCGCGCCCTGGCCTTGGCCCAACG GGTGGAGTCGGGTATCTGCCATATCAACGGCCCGACCGTTCACGATGAAGCGC AGATGCCGTTTGGCGGGGTCAAGTCCAGCGGCTATGGCAGCTTCGGCAGCCGC ACGGCCATCGATCAGTTCACCCAGTTGCGCTGGGTCACCCTCCAGCACGGCCC GCGTCACTATCCCATCTAG
aroY	ATGCAAAACCCCATCAACGATCTCAGAAGCGCCATCGCGTTGCTGCAACGCCA TCCAGGTCACTATATCGAAACCGATCACCCGGTAGATCCCAATGCTGAACTGGC GGGCGTCTACCGCCATATCGGCGCGGGCGGTACCGTAAAACGCCCCACCCGCA CGGGCCCGGCCATGATGTTCAATAGCGTGAAGGGCTACCCTGGCTCCCGCATCC TGGTAGGTATGCACGCCAGCCGGGAAAGAGCGGCGCTTCTGCTGGGCTGTGTA

	CCCTCGAAGCTGGCACAGCACGTTGGTCAGGCGGTGAAAAACCCGGTTGCAC CGGTGGTGGTTCCGGCCTCGCAGGCACCGTGCCAGGAGCAGGTCTTTTACGCC GACGATCCGGACTTTGACCTGCGTAAGCTGCTTCCGGCCCCGACCAACACGCC GATTGATGCAGGCCCGTTCTTCTGTCTGGGGCTGGTACTGGCAAGCGATCCGGA AGATACCTCGCTGACCGATGTGACCATTCACCGTCTCTGTGTGCAGGAGCGAGA TGAACTCTCTATGTTCCTTGCCGCCGGCCGCCATATCGAAGTCTTTCGCAAGAA GGCCGAAGCGGCGGGCAAACCGCTGCCGGTAACCATCAATATGGGACTTGACC CGGCTATCTACATTGGGGCCTGTTTCGAAGCGCCAACCACGCCATTCGGTTACA ACGAGCTTGGCGTTGCCGGGGCATTACGCCAGCAACCGGTGGAGCTGGTACAG GGCGTGGCGGTAAAAGAGAAAGCGATCGCGCGGGCGGAAATCATCATCGAGG GCGAACTGCTTCCCGGCGTGCGCGTAAGAGAAGATCAGCACACCAACACCGG CCACGCCATGCCGGAGTTCCCGGGCTACTGCGGCGAGGCGAATCCGTCTCTGC CGGTGATCAAAGTGAAAGCCGTGACGATGCGAAACCACGCGATCCTGCAGAC GCTGGTGGGCCCGGGCGAAGAGCACACCACGCTTGCCGGTTTGCCGACCGAG GCCAGCATTCGCAACGCGGTCGAAGAGGCCATTCCCGGCTTTCTGCAAAACGT TTACGCCCACACCGCCGGAGGCGGTAAATTCCTCGGCATTTTACAGGTGAAAA AACGCCAGCCGTCAGACGAAGGACGTCAGGGCCAGGCGGCACTTATCGCCCT GGCCACCTATTCCGAGCTGAAAAACATTATCCTCGTGGATGAAGACGTGGATAT CTTCGACAGCGACGATATCCTGTGGGCAATGACCACCCGCATGCAGGGCGATGT GAGCATCACCACGCTTCCGGGGATCCGCGGCCACCAGCTGGATCCGTCGCAGT CACCGGACTACAGCACCTCGATCCGTGGAAACGGCATTTCCTGCAAGACTATCT TCGACTGCACGGTGCCGTGGGCGCTGAAGGCGCGGTTTGAACGGGCGCCGTTC ATGGAGGTTGACCCCACACCGTGGGCGCCGGAGCTGTTCAGCGATAAAAAATA G
BsdB	ATGAAAGCAGAATTCAAGCGTAAAGGAGGGGGCAAAGTGAAACTCGTTGTCG GAATGACAGGGGCAACAGGGGCCATTTTCGGGGTCAGGCTGCTGCAGTGGCTG AAGGCCGCCGGAGTGGAAACCCATCTCGTTGTGTCTCCTTGGGCAAACGTCAC GATCAAACACGAAACAGGCTATACGTTACAAGAAGTAGAACAACTGGCCACAT ACACTTACTCACATAAGGATCAGGCGGCAGCCATTTCAAGCGGGTCGTTTGATA CCGATGGAATGATTGTTGCGCCGTGCAGCATGAAATCTCTCGCAAGCATTCGCA CAGGAATGGCGGATAATCTGCTGACACGTGCGGCGGATGTCATGCTCAAGGAG AGAAAAAAACTCGTCCTCTTAACGAGAGAGACGCCTTTGAACCAAATTCATCT CGAAAATATGCTAGCGCTTACGAAAATGGGCACCATCATTCTTCCTCCGATGCC GGCATTTTATAATCGGCCGAGAAGCTTAGAGGAAATGGTTGACCATATTGTTTTT AGAACGTTGGACCAATTCGGCATTCGGCTTCCTGAAGCGAAGCGCTGGAATGG GATTGAAAAACAAAAAGGAGGAGCTTGA
BsdC	ATGGCTTATCAAGATTTCAGAGAATTTCTCGCTGCCCTTGAAAAAGAAGGACAG CTGCTTACAGTGAATGAAGAGGTAAAGCCGGAACCGGATTTAGGGGCCTCCGC ACGGGCAGCCAGCAATCTTGGCGATAAAAGCCCTGCGCTCTTATTTAACAACAT TTACGGCTATCATAACGCGCGAATTGCGATGAATGTCATCGGCTCTTGGCCAAA CCATGCCATGATGCTGGGCATGCCGAAAGACACACCGGTAAAAGAACAGTTTT TTGAATTCGCAAAGCGTTATGACCAGTTTCCGATGCCGGTCAAACGTGAGGAA ACAGCGCCATTTCATGAAAATGAAATCACAGAAGATATCAATTTGTTCGATATAC TGCCTCTTTTCAGAATTAACCAGGGTGATGGAGGCTACTATTTAGACAAAGCAT

	GTGTCATTTCCCGTGATCTTGAGGACCCTGACAACTTCGGCAAACAAAATGTCG GCATTTACAGAATGCAAGTCAAAGGAAAAGACCGCCTTGGCATTCAGCCTGTC CCGCAGCACGATATTGCAATCCATCTGCGCCAAGCTGAAGAACGCGGCATCAA CCTTCCGGTCACTATTGCGCTCGGCTGTGAGCCGGTCATTACAACGGCGGCATC GACTCCGCTTCTCTATGATCAATCAGAATACGAAATGGCAGGTGCGATTCAAGG CGAACCATATCGCATCGTCAAATCAAAGCTGTCTGATCTTGATGTTCCGTGGGG CGCTGAAGTGGTGCTTGAAGGTGAGATTATTGCCGGAGAGCGCGAATATGAAG GGCCGTTCGGTGAATTCACAGGCCATTATTCCGGCGGACGCAGCATGCCGATTA TCAAAATTAAACGCGTCTATCACAGAAACAATCCGATCTTTGAACATTTATACTT AGGCATGCCTTGGACAGAATGCGATTACATGATCGGCATTAACACATGCGTGCC GCTTTATCAGCAGTTAAAAGAAGCGTATCCGAACGAAATTGTGGCAGTGAACG CCATGTACACACACGGTTTAATCGCGATTGTTTCCACAAAAACCCGCTATGGCG GATTTGCGAAAGCGGTCGGCATGCGCGCACTCACAACGCCGCACGGACTCGGC TACTGCAAAATGGTCATAGTCGTTGATGAGGATGTCGATCCATTCAACCTTCCG CAGGTCATGTGGGCGCTTTCGACCAAAATGCATCCGAAACATGATGCGGTCATC ATTCCGGACTTATCTGTCCTGCCGCTTGATCCGGGATCCAATCCATCAGGAATCA CTCACAAAATGATTCTCGACGCCACTACACCGGTTGCGCCGGAAACAAGAGGC CATTATTCACAGCCGCTTGATTCTCCGCTAACAACGAAAGAATGGGAACAAAA ACTAATGGACTTAATGAATAAATAA
BsdD	ATGCATACATGTCCTCGATGCGACTCAAAAAAGGGAGAAGTCATGAGCAAATC GCCTGTAGAAGGCGCATGGGAAGTTTATCAGTGCCAAACATGCTTTTTTACATG GAGATCCTGTGAACCGGAAAGCATTACAAATCCCGAAAAATACAATCCAGCGT TTAAAATTGATCCAAAGGAAACAGAAACAGCAATTGAAGTTCCGGCGGTGCCG GAACGAAAGGCTTGA
KpdB	ATGAAACTGATTATTGGGATGACGGGGGCCACCGGGGCACCGCTTGGGGTGGC ATTGCTGCAGGCGCTGCGCGATATGCCGGAGGTGGAAACCCATCTGGTGATGTC GAAATGGGCCAAAACCACCATCGAGCTGGAAACGCCCTGGACGGCGCGCGAA GTGGCCGCGCTGGCGGACTTTTCCCACAGCCCGGCAGACCAGGCCGCCACCAT CTCATCCGGTTCATTTCGTACCGACGGCATGATCGTTATTCCCTGCAGTATGAAA ACGCTTGCAGGCATTCGCGCGGGTTATGCCGAAGGGCTGGTGGGCCGCGCGGC GGACGTGGTGCTCAAAGAGGGGCGCAAGCTGGTGTTGGTCCCGCGGGAAATG CCGCTCAGCACGATCCATCTGGAGAACATGCTGGCGCTGTCCCGCATGGGCGT GGCGATGGTCCCGCCCATGCCAGCTTACTACAACCACCCGGAGACGGTTGACG ATATCACCAATCATATCGTCACCCGGGTGCTGGATCAGTTTGGCCTCGACTATCA CAAAGCGCGCCGCTGGAACGGCTTACGCACGGCAGAACAATTTGCACAGGAG ATCGAATAA
KpdC	ATGGCTTTTGATGATTTGCGCAGCTTTTTGCAGGCGCTGGATGACCAGGGACAA CTGCTGAAAATCAGTGAAGAGGTGAACGCTGAGCCCGATCTGGCGGCGGCCGC CAATGCGACCGGACGCATCGGCGACGGTGCCCCGGCGCTGTGGTTCGATAATAT TCGCGGCTTTAACGACGCTCGCGTGACGATGAACACCATCGGCTCGTGGCAGA ACCATGCCATCTCGCTGGGCCTGCCGCCTAACACGCCGGTGAAAAAGCAGATT GATGAGTTCATTCGCCGCTGGGATAACTTCCCGGTGACGCCAGAGCGCCGCGC CAACCCGGCGTGGGCAGAAAATACTGTTGATGGCGATGATATCAACCTGTTCGA TATTCTGCCGCTGTTCCGCCTCAATGATGGTGACGGCGGTTTCTACCTCGATAAA

	GCCTGTGTCGTTTCACGCGATCCGCTGGATAAAGATAACTTCGGTAAGCAAAAC GTCGGTATCTACCGCATGGAAGTGAAAGGCAAGCGCAAGCTCGGCCTGCAGCC GGTACCGATGCACGATATCGCGCTGCATCTGCACAAAGCGGAAGAGCGTGGGG AAGATCTGCCGATCGCCATTACCCTCGGTAACGACCCGATTATTACCCTGATGGG CGCCACGCCGCTGAAATACGATCAGTCTGAATATGAGATGGCTGGCGCGCTGCG CGAGAGCCCGTATCCCATCGCTACCGCGCCGCTGACCGGCTTTGACGTGCCCTG GGGTTCGGAAGTGATCCTCGAAGGGGTCATTGAAGGGCGTAAGCGTGAGATCG AGGGGCCGTTCGGTGAATTTACCGGTCACTACTCCGGCGGTCGTAACATGACG GTAGTGCGTATCGACAAAGTCTCTTATCGCAGCAAACCGATTTTTGAATCGCTCT ACCTCGGTATGCCGTGGACCGAGATTGACTATCTGATGGGCCCGGCGACCTGCG TACCGCTGTATCAGCAGCTGAAGGCCGAATTTCCGGAAGTGCAGGCGGTCAAC GCCATGTACACCCATGGTCTGCTGGCGATCATCTCCACCAAAAAACGCTACGGC GGTTTTGCCCGCGCGGTGGGCCTGCGGGCGATGACCACTCCCCACGGCCTCGG CTATGTGAAGATGGTGATCATGGTTGATGAAGACGTCGACCCGTTCAACCTGCC GCAGGTGATGTGGGCGCTCTCCTCGAAGGTTAACCCGGCGGGAGATCTGGTGC AGTTGCCGAACATGTCGGTCCTTGAACTTGACCCAGGCTCCAGCCCGGCAGGC ATTACCGACAAACTGATTATCGACGCCACCACCCCGGTTGCGCCGGACCTTCGC GGTCACTACAGCCAGCCGGTGCAGGATCTGCCGGAAACCAAAGCCTGGGCTG AAAAACTGACCGCTATGCTGGCCAACCGTAAATAA
KpdD	ATGATTTGTCCACGTTGCGCCGATGAAAAGATTGAAGTCATGGCGACCTCGCCG GTGAAAGGGGTCTGGACCGTGTATCAGTGCCAGCACTGTCTTTACACCTGGCG AGATACCGAGCCGCTGCGCCGCACCAGCCGCGAACACTATCCGGAAGCGTTCC GCATGACGCAGAAAGATATCGATGAGGCGCCGCAGGTGCCGCACGTACCGCCG CTATTGCCGGAAGATAAGCGTTAA
tpl	ATGAATTATCCGGCAGAACCCTTCCGTATTAAAAGCGTTGAAACTGTATCTATGA TCCCGCGTGATGAACGCCTTAAGAAAATGCAGGAAGCGGGATACAATACTTTCC TGTTAAATTCGAAAGATATTTATATTGACCTGCTGACAGACAGTGGCACTAACG CAATGAGCGACAAGCAGTGGGCCGGCATGATGATGGGTGATGAAGCCTACGCG GGCAGCGAAAACTTCTATCATCTGGAAAGAACCGTGCAGGAACTGTTTGGCTT TAAACATATTGTTCCTACTCACCAGGGGCGCGGCGCAGAAAACCTGTTATCGCA GCTGGCAATTAAACCGGGGCAATATGTTGCCGGGAATATGTATTTCACTACTACC CGTTATCACCAGGAAAAAAATGGTGCGGTGTTTGTCGATATCGTTCGTGACGAA GCGCACGATGCCGGTCTGAATATTGCTTTTAAAGGTGATATCGATCTTAAAAAAT TACAAAAACTGATTGATGAAAAAGGCGCCGAGAATATTGCCTATATTTGCCTGG CAGTCACGGTTAACCTCGCAGGCGGGCAGCCGGTTTCCATGGCTAACATGCGC GCGGTGCGTGAACTGACTGCAGCACATGGCATTAAAGTGTTCTACGACGCTAC CCGCTGCGTAGAAAACGCCTACTTTATCAAAGAGCAAGAGCAGGGCTTTGAGA ACAAGAGCATCGCAGAGATCGTGCATGAGATGTTCAGCTACGCCGACGGTTGT ACCATGAGTGGTAAAAAAGACTGTCTGGTGAATATCGGCGGCTTCCTGTGCATG AACGATGACGAAATGTTCTCTTCTGCCAAAGAGTTAGTCGTTGTCTACGAAGGC ATGCCATCTTACGGCGGCCTGGCCGGACGCGACATGGAAGCCATGGCGATTGG TCTGCGCGAAGCCATGCAGTATGAGTACATCGAGCACCGCGTGAAGCAGGTTC GCTATCTGGGCGACAAGCTGAAAGCCGCTGGTGTACCGATTGTTGAACCGGTG GGCGGTCATGCGGTATTCCTCGATGCGCGTCGCTTCTGTGAGCATCTGACGCAG

	GACGAGTTCCCGGCGCAAAGCCTGGCTGCCAGTATCTATGTGGAAACCGGCGT ACGTAGTATGGAGCGCGGAATTATCTCTGCGGGCCGTAATAACGTGACTGGTGA ACACCACAGGCCGAAACTGGAAACCGTGCGTCTGACTATTCCACGCCGCGTTT ATACTTACGCGCATATGGATGTAGTGGCTGACGGTATTATTAAACTTTACCAGCA CAAAGAAGATATTCGCGGGCTGAAGTTTATTTACGAGCCGAAGCAGCTCCGTTT CTTTACTGCACGCTTTGACTATATCTAA
tpl(M379V)	ATGAATTATCCGGCAGAACCCTTCCGTATTAAAAGCGTTGAAACTGTATCTATGA TCCCGCGTGATGAACGCCTTAAGAAAATGCAGGAAGCGGGATACAATACTTTCC TGTTAAATTCGAAAGATATTTATATTGACCTGCTGACAGACAGTGGCACTAACG CAATGAGCGACAAGCAGTGGGCCGGCATGATGATGGGTGATGAAGCCTACGCG GGCAGCGAAAACTTCTATCATCTGGAAAGAACCGTGCAGGAACTGTTTGGCTT TAAACATATTGTTCCTACTCACCAGGGGCGCGGCGCAGAAAACCTGTTATCGCA GCTGGCAATTAAACCGGGGCAATATGTTGCCGGGAATATGTATTTCACTACTACC CGTTATCACCAGGAAAAAAATGGTGCGGTGTTTGTCGATATCGTTCGTGACGAA GCGCACGATGCCGGTCTGAATATTGCTTTTAAAGGTGATATCGATCTTAAAAAAT TACAAAAACTGATTGATGAAAAAGGCGCCGAGAATATTGCCTATATTTGCCTGG CAGTCACGGTTAACCTCGCAGGCGGGCAGCCGGTTTCCATGGCTAACATGCGC GCGGTGCGTGAACTGACTGCAGCACATGGCATTAAAGTGTTCTACGACGCTAC CCGCTGCGTAGAAAACGCCTACTTTATCAAAGAGCAAGAGCAGGGCTTTGAGA ACAAGAGCATCGCAGAGATCGTGCATGAGATGTTCAGCTACGCCGACGGTTGT ACCATGAGTGGTAAAAAAGACTGTCTGGTGAATATCGGCGGCTTCCTGTGCATG AACGATGACGAAATGTTCTCTTCTGCCAAAGAGTTAGTCGTTGTCTACGAAGGC ATGCCATCTTACGGCGGCCTGGCCGGACGCGACATGGAAGCCATGGCGATTGG TCTGCGCGAAGCCATGCAGTATGAGTACATCGAGCACCGCGTGAAGCAGGTTC GCTATCTGGGCGACAAGCTGAAAGCCGCTGGTGTACCGATTGTTGAACCGGTG GGCGGTCATGCGGTATTCCTCGATGCGCGTCGCTTCTGTGAGCATCTGACGCAG GACGAGTTCCCGGCGCAAAGCCTGGCTGCCAGTATCTATGTGGAAACCGGCGT ACGTAGTGTGGAGCGCGGAATTATCTCTGCGGGCCGTAATAACGTGACTGGTGA ACACCACAGGCCGAAACTGGAAACCGTGCGTCTGACTATTCCACGCCGCGTTT ATACTTACGCGCATATGGATGTAGTGGCTGACGGTATTATTAAACTTTACCAGCA CAAAGAAGATATTCGCGGGCTGAAGTTTATTTACGAGCCGAAGCAGCTCCGTTT CTTTACTGCACGCTTTGACTATATCTAA

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