

The catalytic role of glutathione transferases in heterologous anthocyanin biosynthesis

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Supplementary Table 1. List of enzymes used in this study

Abb.	Name	Organism	Anthocyanin ¹ synthesis
AtPAL2	Phenylalanine ammonia lyase	<i>A. thaliana</i>	l-phenylalanine → cinnamic acid + NH ₃
AmC4H	Cinnamate-4-hydroxylase	<i>A. majus</i>	cinnamic acid + redCPR + O ₂ → p-coumaric acid + oxCPR + H ₂ O
AtCPR1	Cytochrome P450 reductase	<i>A. thaliana</i>	oxC4H + NADPH → redC4H + NADP ⁺ + H ⁺
CrCPR1	Cytochrome P450 reductase	<i>C. roseus</i>	oxC4H + NADPH + H ⁺ → redC4H + NADP ⁺
At4CL2	4-coumarate-CoA ligase	<i>A. thaliana</i>	p-coumaric acid + ATP + CoA → p-coumaroyl-CoA + AMP + PP _i
MdCHS2	Chalcone synthase	<i>M. domestica</i>	p-coumaroyl-CoA + 3 malonyl-CoA → naringenin chalcone + 3 CO ₂ + 4 CoA
MsCHI	Chalcone isomerase	<i>M. sativa</i>	naringenin chalcone → naringenin
OsF3'H	Flavonoid-3'-hydroxylase	<i>O. sativa</i>	naringenin + redCPR + O ₂ → eriodictyol + oxCPR + H ₂ O
MdF3H	Flavanone-3-hydroxylase	<i>M. domestica</i>	eriodictyol + 2-oxoglutarate + O ₂ → dihydroquercetin + succinate + CO ₂
PtDFR	Dihydroflavonol-4-reductase	<i>P. trichocarpa</i>	dihydroquercetin + NADPH + H ⁺ → 3,4-cis-leucocyanidin + NADP ⁺
AtLDOX	Leucoanthocyanidin dioxygenase	<i>A. thaliana</i>	3,4-cis-leucocyanidin + 2-oxoglutarate + O ₂ → flavan-3,3,4-triol + succinate + CO ₂
PhLDOX	Leucoanthocyanidin dioxygenase	<i>P. trichocarpa</i>	3,4-cis-leucocyanidin + 2-oxoglutarate + O ₂ → flavan-3,3,4-triol + succinate + CO ₂
ZmBZ2	Glutathione transferases	<i>Z. mays</i>	flavan-3,3,4-triol → cyanidin + H ₂ O
PhAN9	Glutathione transferases	<i>P. hybrida</i>	flavan-3,3,4-triol → cyanidin + H ₂ O
AtTT19	Glutathione transferases	<i>A. thaliana</i>	flavan-3,3,4-triol → cyanidin + H ₂ O
VvGST4	Glutathione transferases	<i>V. vinifera</i>	flavan-3,3,4-triol → cyanidin + H ₂ O
PtGSTF8	Glutathione transferases	<i>P. trichocarpa</i>	flavan-3,3,4-triol → cyanidin + H ₂ O
VvGST1	Glutathione transferases	<i>V. vinifera</i>	-
VvGST2	Glutathione transferases	<i>V. vinifera</i>	-
VvGST3	Glutathione transferases	<i>V. vinifera</i>	-
VvGST5	Glutathione transferases	<i>V. vinifera</i>	-
FaA3GT2	Anthocyanidin-3-O-glycosyltransferase	<i>F. ananassa</i>	cyanidin + UDP-D-glucose → cyanidin-3-O-glucoside + UDP

¹ Molecule names refer to the synthesis of cyanidin-3-O-glucoside.

Supplementary Table 2. List of plasmids used in this study.

Name	Insert	Backbone	Type ¹	Source
pANT1	<i>AtPAL2</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT2	<i>AmC4H</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT3	<i>ScCPR1</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT4	<i>At4CL2</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT5	<i>MdCHS2</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT6	<i>MsCHI</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT8	<i>MdF3H</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT9	<i>PtDFR</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT10	<i>AtCPR1_co1</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT11	Strep-tag- <i>AtLDOX</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT12	Strep-tag- <i>PhLDOX</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT13	<i>FaA3GT2</i>	pTwist Amp MC	GS	Obtained from Twist Bioscience
pANT14	<i>ZmBZ2</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT15	<i>PhAN9</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT16	<i>AtTT19</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT17	<i>VvGST1</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT18	<i>VvGST2</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT19	<i>VvGST3</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT20	<i>VvGST4</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT21	<i>VvGST5</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT22	B/P _{GPD1} - <i>MdCHS2</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone insert of pANT5 into backbone of pHR1 using HindIII and SacII
pANT23	B/P _{GPD1} - <i>AtLDOX</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScAtANS_fw and ScAtANS_rv from template pANT11 into pHR1 using HindIII and SacII
pANT24	B/P _{GPD1} - <i>AtLDOX</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScPhANS_fw and ScPhANS_rv from template pANT12 into pHR1 using HindIII and SacII
pANT25	C/P _{PGK1} - <i>MsCHI</i> -T _{ADH2} /D	pTwist Amp	YAS	Subclone insert of pANT6 into backbone of pHR2 using HindIII and SacII
pANT26	C/P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /D	pTwist Amp	YAS	Subclone insert of pANT13 into backbone of pHR2 using HindIII and SacII
pANT27	D/P _{TEF1} - <i>At4CL2</i> -T _{ENO2} /E	pTwist Amp	YAS	Subclone insert of pANT4 into backbone of pHR10 using HindIII and SacII
pANT28	D/P _{TEF1} - <i>MdF3H</i> -T _{ENO2} /E	pTwist Amp	YAS	Subclone insert of pANT8 into backbone of pHR10 using HindIII and SacII

pANT29	E/P _{PDC1} - <i>AtPAL2</i> -T _{FBA1} /F	pTwist Amp	YAS	Subclone insert of pANT1 into backbone of pHR11 using HindIII and SacII
pANT30	E/P _{PDC1} - <i>PtDFR</i> -T _{FBA1} /F	pTwist Amp	YAS	Subclone insert of pANT9 into backbone of pHR11 using HindIII and SacII
pANT31	F/P _{TEF2} - <i>AmC4H</i> -T _{PGH1} /G	pTwist Amp	YAS	Subclone insert of pANT2 into backbone of pHR12 using HindIII and SacII
pANT33	G/P _{PYK1} - <i>ScCPR1</i> -T _{PDC6} /H	pTwist Amp	YAS	Subclone insert of pANT3 into backbone of pHR13 using HindIII and SacII
pANT35	<i>PtGSTF8</i>	pET-28b(+)	BEX	Obtained from Twist Bioscience
pANT40	G/P _{PYK1} - <i>ScCPR1</i> -T _{ADH1} /H	pTwist Amp	YAS	Subclone PCR product obtained with primers tADH_fw and tADH_rv from <i>S. cerevisiae</i> S288C genomic DNA into pANT33 using SacII and SphI
pANT43	<i>AtLDOX</i> -Strep-tag	pET-28b(+)	BEX	
pANT46	G/P _{PYK1} - <i>ScCPR1</i> -T _{ADH1} /Z	pTwist Amp	YAS	Subclone insert of pANT40 into backbone of pHR7 using SacI and SphI
pANT54	G/P _{PYK1} - <i>AtCPR1</i> _co1-T _{ADH1} /Z	pTwist Amp	YAS	Subclone insert of pANT10 into backbone of pHR46 using HindIII and SacII
pANT55	E/P _{PDC1} - <i>PtDFR</i> -T _{ADH1} /Z	pTwist Amp	YAS	Subclone insert of pANT30 into backbone of pANT46 using SacI and SacII
pANT56	D/P _{TEF1} - <i>AtCPR1</i> _co1-T _{ADH1} /Z	pTwist Amp	YAS	Subclone insert of pANT54 into backbone of pHR10 using HindIII and BamHI
pANT57	B/P _{GPD1} - <i>ZmBZ2</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScZmBZ2_fw and ScZmBZ2_rv from template pANT14 into pHR1 using HindIII and SacII
pANT58	B/P _{GPD1} - <i>PhAN9</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScPhAN9_fw and ScPhAN9_rv from template pANT15 into pHR1 using HindIII and SacII
pANT59	B/P _{GPD1} - <i>AtTT19</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScAtTT19_fw and ScAtTT19_rv from template pANT16 into pHR1 using HindIII and SacII
pANT62	<i>PtGSTF8</i> (C13S)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pANT70	B/P _{GPD1} - <i>VvGST1</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScVvGST1_fw and ScVvGST1_rv from template pANT17 into pHR1 using HindIII and SacII
pANT71	B/P _{GPD1} - <i>VvGST2</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScVvGST2_fw and ScVvGST2_rv from template pANT18 into pHR1 using HindIII and SacII
pANT72	B/P _{GPD1} - <i>VvGST3</i> -T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScVvGST3_fw and

pANT73	B/P _{GPD1} -VvGST4-T _{TPS1} /C	pTwist Amp	YAS	ScVvGST3_rv from template pANT19 into pHR1 using HindIII and SacII
pANT74	B/P _{GPD1} -VvGST5-T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScVvGST4_fw and ScVvGST4_rv from template pANT20 into pHR1 using HindIII and SacII
pANT75	B/P _{GPD1} -PtGSTF8-T _{TPS1} /C	pTwist Amp	YAS	Subclone PCR product obtained with primers ScVvGST5_fw and ScVvGST5_rv from template pANT21 into pHR1 using HindIII and SacII
pANT78	C/P _{PGK1} -OsF3'H-T _{ADH2} /D	pTwist Amp	YAS	Subclone PCR product obtained with primers ScPtGSTF8_fw and ScPtGSTF8_rv from template pANT35 into pHR1 using HindIII and SacII
pANT80	D/P _{TEF1} -CrCPR-T _{ADH1} /Z	pTwist Amp	YAS	Subclone PCR product obtained with primers T7 fw and T7 term from template OsF3'H_gB into pHR2 using HindIII and SacII
pANT81	G/P _{PYK1} -AtCPR1_co2-T _{ADH1} /Z	pTwist Amp	YAS	Subclone DNA fragment CrCPR into pANT56 using HindIII and SacII
pANT86	D/P _{TEF1} -PhLDOX-T _{ENO2} /E	pTwist Amp	YAS	Subclone DNA fragment AtCPR1_co2 into pHR46 using HindIII and SacII
pANT95	AtGSTF2	pET-28b(+)	BEX	Subclone DNA fragment PhLDOX into pHR10 using HindIII and SacII
pHLUM	LEU2/URA3/HIS3/MET17	pRS313	YEX	Obtained from Twist Bioscience
pHR1	B/P _{GPD1} -T _{TPS1} /C	pTwist Amp	YAS	Obtained from Addgene
pHR2	C/P _{PGK1} -T _{ADH2} /D	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR3	D/T _{ENO2} /E	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR4	E/T _{FBA1} /F	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR5	F/T _{PGI1} /G	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR6	G/T _{PDC6} /H	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR7	H/Z	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR8	Z/A	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR9	A/B	pTwist Amp	YAS	Obtained from Twist Bioscience
pHR10	D/P _{TEF1} -T _{ENO2} /E	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT1 and ANT2 from <i>S. cerevisiae</i> S288C genomic DNA into pHR3 using XhoI and HindIII
pHR11	E/P _{PDC1} -T _{FBA1} /F	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT3 and ANT4 from <i>S. cerevisiae</i> S288C genomic DNA into pHR4 using XhoI and HindIII
pHR12	F/P _{TEF2} -T _{PGI1} /G	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT5 and ANT6 from <i>S. cerevisiae</i> S288C genomic DNA into pHR5 using XhoI and HindIII
pHR13	G/P _{PYK1} -T _{PDC6} /H	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT7 and ANT8 from <i>S. cerevisiae</i> S288C genomic DNA into pHR6 using XhoI and HindIII

pHR14	Z/ARS/CEN/A	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT9 and ANT10 from template pHLUM into pHR8 using XhoI and SphI
pHR15	A/HIS3/B	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT11 and ANT12 from template pHLUM into pHR9 using XhoI and SphI
pHR16	A/URA3/B	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT13 and ANT14 from template pHLUM into pHR9 using XhoI and SphI
pHR17	A/LEU2/B	pTwist Amp	YAS	Subclone PCR product obtained with primers ANT15 and ANT16 from template pHLUM into pHR9 using XhoI and SphI
pJK1	<i>PhAN9</i> (A12M)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK2	<i>PhAN9</i> (C13S)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK3	<i>PhAN9</i> (N108H)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK7	<i>VvGST4</i> (A12M)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK8	<i>VvGST4</i> (C13S)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK9	<i>VvGST4</i> (N108H)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK1	<i>PhAN9</i> (A12M)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK2	<i>PhAN9</i> (C13S)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI
pJK3	<i>PhAN9</i> (N108H)	pET-28b(+)	BEX	Subclone overlap extension PCR fragment (see methods section) into pANT35 using NdeI and XhoI

¹ GS: gene synthesis plasmid, BEX: bacterial expression plasmid, YEX: yeast expression plasmid, YAS: yeast assembly plasmid

Supplementary Table 3. List of DNA fragments used in this study.

Name	Content	Source
<i>AtCPR1_co2_F1</i>	<i>AtCPR1_co2</i> fragment 1	Obtained from Twist Bioscience
<i>AtCPR1_co2_F2</i>	<i>AtCPR1_co2</i> fragment 2	Obtained from Twist Bioscience
<i>AtCPR1_co2</i>	<i>AtCPR1_co2</i>	Assembly of fragments <i>AtCPR1_co2_F1</i> and <i>AtCPR1_co2_F2</i> using overlap extension PCR with primers T7 fw, T7 term, <i>AtCPR1_co2_OL_rv</i> , and <i>AtCPR1_co2_OL_fw</i>
<i>CrCPR_F1</i>	<i>CrCPR</i> fragment 1	Obtained from Twist Bioscience
<i>CrCPR_F2</i>	<i>CrCPR</i> fragment 2	Obtained from Twist Bioscience
<i>CrCPR</i>	<i>CrCPR1</i>	Assembly of fragments <i>CrCPR_F1</i> and <i>CrCPR_F2</i> using overlap extension PCR with primers T7 fw, T7 term, <i>CrCPR_OL_rv</i> , and <i>CrCPR_OL_fw</i>
<i>OsF3'H</i>	<i>OsF3'H</i>	Obtained from Twist Bioscience

Supplementary Table 4. List of primers used in this study.

Name	Sequence
ANT1	AGCTACTCGAGTAGCTTCAAAATGTTTCTACTCC
ANT2	AGCTAAAGCTTTTTGTAAATTAACCTTAGATTAGATTGC
ANT3	AGCTACTCGAGATGCGACTGGGTGAGCATATG
ANT4	AGCTAAAGCTTTTTGATAGATTTGACTGTGTTATTTTGCCTGAG
ANT5	AGCTACTCGAGGGGCGGTATACTTACATATAGTAG
ANT6	AGCTAAAGCTTGTTTAGTTAATTATAGTTCGTTGACCG
ANT7	AGCTACTCGAGAATGCTAGTATTTTGAGATTAAATCTC
ANT8	AGCTAAAGCTTTGTGATGATGTTTTATTTGTTTTGATTG
<i>AtCPR1_co2_OL_fw</i>	GGTATCACTTACGAAACAGGTG
<i>AtCPR1_co2_OL_rv</i>	CACCTGTTTCGTAAGTGATACC
<i>C_Strep_AtANS_rv</i>	AGCTACTCGAGTTACTTTTCGAACTGCGGGTGGCTCCATGCGCTATCGTTCCTTTT CGGAAACCAATTC
C13S_fw	GTTTATGGTCCGGCAGTTGCAGTTAGCCCGCAGCGTGTTATGG
C13X_fw	GTTTATGGTCCGGCAGTTGCAGTTNNKCCACAGCGTGTTATGGCATGTC
<i>CrCPR_OL_fw</i>	GGAACAGGTGATCATGTAGG
<i>CrCPR_OL_rv</i>	CCTACATGATCACCTGTTCC
F112_rv	CACCAGGTCATTGAAATTATGTG
F112X_fw	CACATAATTTCAATGACCTGGTGNNKAATATTGTGTTTCAGGTTGTTATTCTG
<i>MET15</i> outer fw	GGGTTCGAATCCCTTAGCTCTC
<i>MET15</i> outer rv	ACCAACTGGGCCAAGAGACC
<i>MET15_fw</i>	AGGTCACATGATCGCAAAATGGC
<i>MET15_rv</i>	GGACATATTAACTATGACGACATTGTTGC
N108_rv	GAAATTATGTGCTTCAATTTCCAGC
N108X_fw	GCTGGAAATTGAAGCACATAATTTCNNKGATCTGGTGTTCAACATTGTGTTTC
<i>NdeI_AtANS_fw</i>	AGCTACATATGGTTGCCGTTGAAAGAG
<i>PhAN9_A12M_fw</i>	GTTTCATGGTTCTGCTATGGCTATGTGTCCACAAAGAGTTATGGTTTG
<i>PhAN9_A12M_rv</i>	AGCCATAGCAGAACCATGAAC
<i>PhAN9_C13S_fw</i>	GGTTCGCTATGGCTGCTAGCCACAAAGAGTTATGGTTTGC
<i>PhAN9_C13S_rv</i>	AGCAGCCATAGCAGAACC
<i>PhAN9_N108H_fw</i>	GTTGGAAGTCGAATCTAACAACCTACCATGATCTGGTTTACAACATGGTGTTG
<i>PhAN9_N108H_rv</i>	GTAGTTGTTAGATTTCGACTTCCAAC
<i>PtGSTF8_rv</i>	AGCTACCGCGGTTAAAAACCTGCCAGGTTTCATC
<i>ScAtANS_fw</i>	AGCTAAAGCTTAAAAATGGTTGCCGTTGAAAGAG
<i>ScAtANS_rv</i>	AGCTACCGCGGTTAATCGTTCCTTTTCGGAAC
<i>ScAtTT19_fw</i>	AGCTAAAGCTTAAAAATGGTCGTTAAGTTGTACGG
<i>ScAtTT19_rv</i>	AGCTACCGCGGTCAGTGACCAGCCAAAAC
<i>ScPhAN9_fw</i>	AGCTAAAGCTTAAAAATGGTTGTTAAGGTTTCATGGTTC
<i>ScPhAN9_rv</i>	AGCTACCGCGGTCAGACCTTAGCTTCTTCTTTAG
<i>ScPhANS_fw</i>	AGCTAAAGCTTAAAAATGGTTAACGCTGTTGTTAC
<i>ScPhANS_rv</i>	AGCTACCGCGGTTACTTAGATTCTTCAGCGGC
<i>ScPtGSTF8_fw</i>	AGCTAAAGCTTAAAAATGGTGGTGAAAGTTTATGGTC
<i>ScVvGST1_fw</i>	AGCTAAAGCTTAAAAATGGCCAACCTCTGATCATATC
<i>ScVvGST1_rv</i>	AGCTACCGCGGTCAGATGCCCATCTTCTTTTC
<i>ScVvGST2_fw</i>	AGCTAAAGCTTAAAAATGGCCGTTTTGAAAGTTC

<i>ScVvGST2_rv</i>	AGCTACCGCGGTTAGGACTTTTGCATAGCAATAAC
<i>ScVvGST3_fw</i>	AGCTAAAGCTTAAAAATGGTTGTTAAGGTTTACGGTC
<i>ScVvGST3_rv</i>	AGCTACCGCGGTCACTCCAATAATGGCCATC
<i>ScVvGST4_fw</i>	AGCTAAAGCTTAAAAATGGTCATGAAGGTTTATGGTC
<i>ScVvGST4_rv</i>	AGCTACCGCGGTTAAGCAGCCAATTCCATGAC
<i>ScVvGST5_fw</i>	AGCTAAAGCTTAAAAATGGCCGACGAAATTATCTTG
<i>ScVvGST5_rv</i>	AGCTACCGCGGTACTCGATACCGAATCTCTTTC
<i>ScZmBZ2_fw</i>	AGCTAAAGCTTAAAAATGACTGCTGGTACTATGAGAG
<i>ScZmBZ2_rv</i>	AGCTACCGCGGTCAAGAAACGTGGACTCTG
T7 fw	TAATACGACTCACTATAGGG
T7 term	GCTAGTTATTGCTCAGCGG
tADH_fw	AGCTACCGCGGGCTTTGGACTTCTTCGCC
tADH_rv	AGCTAGCATGCGGTAGAGGTGTGGTCAATAAGAG
V12C13_rv	AACTGCCGGACCATAAAC
V12X_fw	GTTTATGGTCCGGCAGTTGCANNKTGCCCAGCGTGTTATGG
VvGST4_A12M_fw	GAAGGTTTATGGTCCAGTTAGAGCTATGTGTCCACAAAGAGTTTGGC
VvGST4_A12M_rv	AGCTCTAACTGGACCATAAACCTTC
VvGST4_C13S_fw	GTTTATGGTCCAGTTAGAGCTGCTAGCCCACAAAGAGTTTGGCTTG
VvGST4_C13S_rv	AGCAGCTCTAACTGGACCATAAAC
VvGST4_N108H_fw	GTTGGAAGTTGAAGCTCACAACTTTCATGAATTGGTTTACACCTTGGTCATG
VvGST4_N108H_rv	AAAGTTGTGAGCTTCAACTTCCAAC

Supplementary Table 5. List of yeast strains used in this study.

Name	Genotype	Construction
S288C	<i>MATa SUC2 gal2 mal2 mel flo1 flo8-1 hap1 ho bio1 bio6</i>	Obtained from NCYC
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Obtained from Euroscarf
BY4741_ULH	<i>MATa his3Δ1 leu2Δ0 ura3Δ0</i>	Integration of PCR product with primers MET15_fw and MET15_rv from genomic DNA of <i>S. cerevisiae</i> S288C
NAR	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} - <i>MdCHS2</i> -T _{TPS1} /P _{PGK1} - <i>MsCHI</i> -T _{ADH2} /P _{TEF1} - <i>At4CL2</i> -T _{ENO2} /P _{PDC1} - <i>AtPAL2</i> -T _{FBA1} /P _{TEF2} - <i>AmC4H</i> -T _{PGI1} /P _{PYK1} - <i>AtCPR1</i> -co2-T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT22, pANT25, pANT27, pNAT29, pANT31, pANT81, pHR14, pHR16
P3G	NAR [ARS/CEN/HIS3/P _{GPD1} - <i>AtLDOX</i> -T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>MdF3H</i> -T _{ENO2} /P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT23, pANT26, pANT28, pANT55, pHR14, pHR15
C3G1	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>ZmBZ2</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT57, pANT78, pANT80, pHR14, pHR17
C3G2	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>PhAN9</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT58, pANT78, pANT80, pHR14, pHR17
C3G3	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>AtTT19</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT59, pANT78, pANT80, pHR14, pHR17
C3G4	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>VvGST4</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT73, pANT78, pANT80, pHR14, pHR17
C3G5	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>PtGSTF8</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT75, pANT78, pANT80, pHR14, pHR17
C3G6	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>VvGST1</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT70, pANT78, pANT80, pHR14, pHR17
C3G7	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>VvGST2</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT71, pANT78, pANT80, pHR14, pHR17
C3G8	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>VvGST3</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT72, pANT78, pANT80, pHR14, pHR17
C3G9	P3G [ARS/CEN/LEU2/P _{GPD1} - <i>VvGST5</i> -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT74, pANT78, pANT80, pHR14, pHR17
C3G10	P3G [ARS/CEN/LEU2/P _{GPD1} -T _{TPS1} /P _{PGK1} - <i>OsF3'H</i> -T _{ADH2} /P _{TEF1} - <i>CrCPR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pHR1, pANT78, pANT80, pHR14, pHR17
DHQC3G1	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} - <i>ZmBZ2</i> -T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} /P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT57, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G2	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} - <i>PhAN9</i> -T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} /P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT58, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G3	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} - <i>AtTT19</i> -T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} /P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT59, pANT26, pANT86, pANT55, pHR14, pHR16

DHQC3G4	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -VvGST4- T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT73, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G5	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} - <i>PtGSTF8</i> - T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT75, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G6	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -VvGST1- T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT70, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G7	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -VvGST2- T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT71, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G8	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -VvGST3- T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT72, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G9	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -VvGST5- T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pANT74, pANT26, pANT86, pANT55, pHR14, pHR16
DHQC3G10	BY_4741_ULH [ARS/CEN/URA3/P _{GPD1} -T _{TPS1} /P _{PGK1} - <i>FaA3GT2</i> -T _{ADH2} /P _{TEF1} - <i>PhLDOX</i> -T _{ENO2} / P _{PDC1} - <i>PtDFR</i> -T _{ADH1}]	Assembly of multi-expression plasmid from <i>AscI</i> digested pHR1, pANT26, pANT86, pANT55, pHR14, pHR16

Supplementary Table 6. Coding sequences of enzymes in this study

Gene	Coding sequence
<i>AmC4H</i>	ATGATGGACTTCGTCTTGTGGAAAAGGCTTTGTTGGGTTTGTTCATTGCTACCATCGTTGCC ATTACCATCTCTAAATTGAGAGGCAAGAACTGAAATTGCCACCAGGTCCAATTCCAGTTCCA GTTTTTGGTAATTGGTTGCAAGTTGGTGATGACTTGAATCAGAGAACTTGGTTGAATACGCC AAAAAGTTCCGGTGACTTGTCTTGTGAGAAATGGGTCAAAGAAATTTGGTCGTTGTTTCCTCA CCAGATTTGGCTAAAGATGTCTTGCATACTCAAGGTGTTGAATTCGGTTCAGAACTAGAAAT GTTGTGTTTCGATATTTTCACCGGTAAGGGTCAAGATATGGTTTTCACTGTTTACTCCGAACAT TGGAGAAAGATGAGAAGAATTATGACGGTTCCATTCTTCACCAACAAGGTTGTTCAACAGTAT AGATTCCGGTTGGGAAGATGAAGCTGCTAGAGTTGTTGAAGATGTTAAGGCTAATCCTGAAGCT GCAACTAATGGTATCGTTTTTGAGAAACAGACTGCAGCTGCTGATGTACAACAACATGTACAGA ATCATGTTTCGACAGAAGGTTTCAATCTGTTGATGATCCTTTGTTCTTGAAGTTGAAGGCTTTG AACGGTGAAAGATCAAGATTGGCTCAATCCTTTGAGTACAACCTTCGGTGATTTTCATCCCAATT TTGAGGCCATTTTTGAGGGGCTACTTGAAATTGTGTCAAGAGATCAAGGACAAGAGGCTGAAG TTGTTTAAGGATTACTTTGTGACGAGAGGGAAGAGTTGGAATCCATTAAGTCTGTTGGCAAC AACTCCTTGAAGTGCGCTATTGATCATATTATCGAAGCCCAAGAAAAGGGTGAATCAATGAA GATAACGTCTTGTACATCGTCGAGAACATTAACGTTGCTGCTATTGAACTACCTTGTGGTCT ATTGAATGGGGTATTGCTGAATTGGTTAACAACCCAGAAATCCAGAAAAAGTTGAGACACGAA TTGGATACCGTTTTAGGTGCTGGTGTCAAATTTGTGAACCAGATGTTCAAAAGCTGCCATAC TTGCAAGCTGTTATCAAAGAAACCTTGAGATACAGAATGGCTATCCCTTTGTTGGTTCCACAC ATGAACCTTGCATGAAGCTAAATTGGCTGGTTACGATATTCCAGCCGAATCCAAGATTTTGGTT AATGCTTGGTGGTTGGCTAACAATCCAGCTCATTGGAACAAACCAGATGAATTGAGACCAGAA AGGTTCTTGGGAAGAAGATCTAAGGTTGAAGCTAACGGTAACGACTTCAAGTACATTCCATTT GGTGTGGTAGAAGATCATGCCCAGGTATTATCTTGGCTTTGCCAATTTTGGGTATCGTCATA GGTAGATTGGTTTCAAGATTTTCGAGTTGTTACCACCACCTGGTCAATCTAAAATTGATACCGCT GAAAAAGGTGGCCAATTCTCCTTGCAAATCTTGAAACATTCTACGATTGTCTGCAAGCCAAGG TCCTCTTAA
<i>At4CL2</i>	ATGACTACCCAAGATGTTATCGTCAACGATCAAAAACGACCAAAAGCAATGTTCCAACGATGTC ATCTTCAGATCAAGATTGCCAGATATCTACATCCCAAACCAATTTGCCATTGCACGATTACATC TTCGAAAACATTTCTGAATTTCGCTGCTAAGCCATGCTTGATTAACGGTCCAACCTGGTGAAGTT TACACTTACGCTGATGTTTCATGTTACCTCCAGAAAAATTTGGCTGCTGGTTTACACAATTTGGGT GTTAAGCAACACGATGTCGTTATGATTTTGTGGCCAACTCTCCAGAAGTTGTCTTGACTTTT TTGGCTGCTTCTTTCATTGGTGCTATTACTACTTCTGCTAACCCTTTTTTACCCAGCCGAA ATTTCTAAACAAGCTAAGGCTTCTGCTGCCAAGTTGATAGTTACTCAATCAAGATACGTCGAC AAGATCAAGAACTTGCAAAACGATGGTGTGTTGATTGTCACCACTGATTCTGATGCTATTCCA GAAACTGCTTGAGGTTCTCTGAATTGACTCAATCTGAAGAACCTAGGGTTGATTCCATTCCA GAGAAAATTTTACCAGAAGATGTTGTTGCTTTGCCCTTTTCTTCTGGTACTACTGGTTTGCCA AAAGGTGTTATGTTGACTCACAAAGGTTTGGTTACATCCGTTGCTCAACAAGTTGATGGTGAA AATCCAAACTTGTACTTCAACAGGGATGACGTTATTTTGTGCGTTTTTGCCAATGTTTCATATC TACGCCTTGAACCTCCATCATGTTGTGTTCTTTGAGAGTTGGTGCCACCATTGATTATGCCA AAGTTTCGAAATCACCTGTTGTTGGAACAAAATCCAAAGATGTAAGGTTACCGTTGCTATGGTT GTTCCACCAATAGTTTTGGCTATTGCTAAGTCTCCAGAAACCGAAAAGTACGATTTGTCTCT GTTAGAATGGTTAAGTCTGGTGCTGCTCCATTGGGTAAAGAATTGGAAGATGCTATTTCTGCT AAGTTCCCAAACGCTAAATTAGGTCAAGGTTACGGTATGACTGAAGCTGGTCCAGTTTTAGCT ATGTCTTTGGGTTTTGCTAAAGAGCCATTTCCAGTAAATCTGGTGCTTGTGGTACTGTTGTT AGAAACCTGAGATGAAGATTTTGGACCCAGATACTGGTGATTCTTTGCCAAGAAACAAACCA GGTGAATCTGCATCAGAGGTAACCAGATTATGAAGGGTTACTTGAATGATCCATTGGCTACT GCTTCTACCATTGATAAGGATGGTTGGTTGCATACAGGTGATGTTGGTTTTCATAGATGATGAT GACGAGTTGTTTCATCGTCGACAGATTGAAAGAACTGATCAAGTACAAGGGTTTCCAAGTTGCT CCAGCTGAATTGGAATCTTTGTTGATTGGTCATCCAGAAATCAACGATGTTGCTGTTGTTGCA ATGAAGGAAGAGGACGCTGGCGAAGTTCCAGTTGCTTTGTTGTTAGATCAAAGGACTCTAAT ATCTCCGAGGACGAAATCAAGCAATTCTGTTTCAAAGCAAGTCGTGTTCTACAAGAGAATCAAC AAGTTTTTCTTACCAGACTCTATTCCAAAAGCTCCATCTGGTAAGATTTTGAGAAAGGATTTG AGAGCTAGATTGGCCAACGGTTTGATGAACATA
<i>AtCPR1_co1</i>	ATGACTTCTGCCTTGTATGCCTCTGATTTTGTTCAGCAATTGAAGTCCATTATGGGCACCGAT TCTTTGTCTGATGATGTTGTTTTGGTTATCGCTACTACCTCTTTGGCTTTGGTTGCTGGTTTT GTTGTTCTGTTGTGGAAAAAGACTACCGCTGATAGATCAGGTGAATTGAAACCATGATGATC CCCAAATCTTTGATGGCCAAAAGATGAAGATGATGACTTGGACTTAGGTTCTGGTAAGACTAGA GTTTCCATTTTCTTCGGTACTCAAACCTGGTACTGCTGAAGGTTTTGCTAAGGCTTTATCTGAA GAAATCAAGGCCAGATACGAAAAGGCTGCTGTTAAGGTTATTGATTTGGATGATTACGCTGCC GATGATGACCAATACGAAGAAAAGTTGAAGAAAAGAAACCTTGCCCTTCTTCTGTGTTGCTACT

TATGGTGATGGTGAACCTACTGATAATGCTGCTAGATTTTACAAGTGGTTCACCGAAGAGAAC
 GAAAGAGATATCAAGTTGCAACAATTGGCCTACGGTGTTTTTGCTTTGGGTAAATAGACAATAC
 GAGCACTTCAACAAGATCGGTATCGTTTTGGATGAAGAGTTGTGTAAAAAGGGTGCCAAGAGA
 TTGATTGAAGTTGGTTTTGGGTGATGACGACCAATCTATCGAAGATGATTTTAAACGCCTGGAAA
 GAATCCTTGTGGTCTGAATTGGATAAGTTGTTGAAGGACGAAGATGACAAATCTGTTGCTACA
 CCATACACTGCTGTTATTCCAGAGTATAGAGTTGTTACTCACGATCCAAGATTACAGACTCAA
 AAGTCTATGGAATCTAACGTTGCTAACGGTAACACCACCATCGATATTCATCATCCATGTAGA
 GTTGATGTCGCCGTCCAAAAAGAATTGCATACTCATGAATCCGATAGGTCCTGCATTCAATTTG
 GAATTCGATATTTCCAGAACCGGTATTACTTACGAAACCGGTGATCATGTTGGTGTTTACGCT
 GAAATCACGTTGAAATCGTTGAAGAAGCCGGTAAGTTGTTAGGTCATTTCATTGGATTGGGTG
 TTCTCCATTCATGCCGACAAAGAAGATGGTTCTCCTTTGGAAATCTGCTGTTCCACCACCATTT
 CCAGGTCCATGTACTTTAGGTACTGGTTTTGGCTAGATATGCTGACTTGTGTAATCCACCAAGA
 AAGTCTGCTTTAGTTGCTTTGGCTGCTTATGCTACTGAACCATCTGAAGCCGAAAAATTGAAA
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 TTGTTGGAAGTTATGGCTGCTTTTCCATCTGCTAAACCACCATTGGGTGTTTTTTTTTGCTGCT
 ATTGCTCCAAGATTGCAACCTAGGTATTACTCCATTTCTTCATCACCAAGATTGGCCCCATCA
 AGAGTTCATGTTACATCTGCTTTGGTTTATGGTCCAACCTCCAACCTGGTAGAATTCATAAGGGT
 GTTTGTTCTACCTGGATGAAGAACGCTGTTCCAGCTGAAAAATCTCATGAATGTTCTGGTGCC
 CCAATTTTCATTAGAGCTTCTAATTTCAAGCTGCCAAGCAATCCATCTACTCCAATAGTTATG
 GTTGGTCCAGGTACAGGTTTAGCTCCTTTTAGAGGTTTCCCTACAAGAAAGGATGGCCTTGAAA
 GAGGATGGCGAAGAATTGGGTTCTTCTGTTGTTTTTGGTTGCAGAAACAGACAGATGGAT
 TTCATCTATGAGGACGAGTTGAACAACCTTCGTTGATCAAGGTGTTATCTCCGAATTGATTATG
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 CAAGTTTGGGACCTAATCAAAGAAGAAGGATACTTGTACGTTTGCAGGTGATGCTAAAGGTATG
 GCTAGAGATGTTCATAGAACATTGCATACCATCGTCCAAGAACAAGAAGGTGTTTCATCTTCT
 GAAGCTGAAGCTATCGTTAAGAAGTTGCAAACTGAAGGTAGATACTTGAGAGATGCTGCTGGA
 ATGACTTCTGCACCTTTATGCCTCCGATCTTTTCAAACAATTGAAAAATCATCAAGGATCGGTA
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 GTTGTCTTATTGTGGAAGAACACCGGCAGATCGTTCCGGCGAGCTAAAGCCACTAATGATC
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 GATGATGACCAATATGAGGAAAAGTTGAAAAAGGAAACATTGGCTTTCTTTTGTGTAGCCACG
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 GAAAGAGATATCAAGTTGCAGCAACTTGCTTACGGCGTTTTTGCCTTAGGTAACAGACAATAC
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 TTGATTGAAGTCGGTTTTAGGAGATGATGATCAATCTATCGAGGATGACTTTAATGCATGGAAG
 GAATCTTTGTGGTCTGAATTAGATAAGTTACTTAAAGGACGAAGATGATAAATCCGTTGCCACT
 CCATACACAGCCGTCAATCCAGAATATAGAGTAGTTACTCATGATCCAAGATTACACAACACAG
 AAATCAATGGAAAGTAATGTGGCTAATGGTAATACTACCATCGATATTCATCATCCATGTAGA
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 GAATTTGATATATCACGTACTGGTATCACTTACGAAACAGGTGATCACGTGGGTGTCTACGCT
 GAAAACCATGTTGAAATTGTAGAGGAAGCTGGAAAGTTGTTGGGCCATAGTTTAGATCTTGTT
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 CCAGGACCATGCACCCTAGGTACCGGTTTAGCTCGTTACGCGGATCTGTTAAATCCTCCACGT
 AAATCAGCTCTAGTGGCCTTGGCTGCGTACGCCACAGAACCTTCTGAGGCAGAAAAACTGAAA
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 AGAGTTCATGTCACATCCGCTTTAGTGTACGGTCCAACCTCTACTGGTAGAATCCATAAGGGC
 GTTTGTTCAACATGGATGAAAAACGCGGTTCCAGCAGAGAAAGTCTCACGAATGTTCTGGTGCT
 CCAATCTTTATCAGAGCCTCCAACCTCAAACCTGCCTTCCAATCCTTCTACTCCTATTGTCATG
 GTCGGTCCCTGGTACAGGTCTTGCTCCATTACAGAGTTTCTTACAAGAGAGAATGGCCTTAAAG
 GAGGATGGTGAAGAGTTGGGATCTTCTTTGTGTTTTTCGGCTGTAGAAACAGACAAATGGAT
 TTCATCTACGAAGATGAACTGAATAACTTTGTAGATCAAGGAGTTATTTTCAGAGTTGATAATG
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AAAGAACCGTTTCTGAGCCGTAATCCGTTTGGTCAGGTTCCGGCATTGTAAGATGGTGATCTGAACTGTTTGAAAGCCGTGCAATTACCCAGTATATTGCCCATCGTTATGAAAATCAGGGCACC
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GTGCAGTAA

AtLDOX ATGGTTGCCGTTGAAAGAGTTGAATCTTTGGCTAAGTCCGGTATTATCTCCATTCCAAAAGAGTACATCAGGCCCCAAAGAAGAAATTGGAATCCATCAACGATGTGTTCTTGGAAGAAAAGAAAGAA
GATGGTCCACAAGTTCCAACCATCGATTTGAAGAACATTGAATCCGATGACGAGAAGATCAGAGAAAAGTGCATTGAGGAATTGAAGGAAAGGTTAAGAAAGCTGTTCTTTGGATTGGGGTGT
TATGCATTTGATTAAACACGGTATTCCAGCCGATTTGATGGAAGGGTTAAGAAAGCTGGTGAAGAGTTCTTCTCATTTGTCGGTTCGAAGAAAAAGAAAAGTACGCTAACGATCAAGCCACCGGTAA
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ATTACATTGAAGCTACATCTGAATACGCTAAGTGCTTGAGATTATTGGCTACCAAGGTTTTTCAAGGCTTTGTCTGTTGGTTTGGGTTTTAGAACCCAGACAGGTTGGAAAAAGAGTTGGAGGTTT
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ACCAAGAAGTTTCGCCCAAACATATCGAACATAAGTTGTTCCGTTAAAGAGCAAGAGGAATTGGTTTCCGAAAAAGAACGATTAA

Strep-tag-AtLDOX ATGGCAAGCTGGAGCCACCCGAGTTTCGAAAAGGGTGCAATGGTTGCCGTTGAAAGAGTTGAATCTTTGGCTAAGTCCGGTATTATCTCCATTCCAAAAGAGTACATCAGGCCCCAAAGAAGAAATTG
GAATCCATCAACGATGTGTTCTTGGAAGAAAAAGAAAGAGATGGTCCACAAGTTCCAACCATC
GATTTGAAGAACATTGAATCCGATGACGAGAAGATCAGAGAAAAGTGCATTGAGGAATTGAAG
AAGGCTTCTTTGGATTGGGGTGTATGCATTTGATTAAACACGGTATTCCAGCCGATTTGATG
GAAAGGGTTAAGAAAGCTGGTGAAGAGTTCTTCTCATTGTCCGTCGAAGAAAAAGAAAAGTAC
GCTAACGATCAAGCCACCGGTAAATTTCAAGGTTACGTTCTAAATTGGCTAACAACGCTTCT
GGTCAATTGGAATGGGAAGATTACTTTTTCCATTTGGCTTACCCAGAAGAGAAGAGAGATTG
TCTATTTGGCCTAAGACTCCATCCGATTACATTGAAGCTACATCTGAATACGCTAAGTGCTTG
AGATTATTGGCTACCAAGGTTTTTCAAGGCTTTGTCTGTTGGTTTGGGTTTTAGAACCCAGACAGG
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AAGTGTCACAACCCAGAATTGGCTTTGGGTGTTGAAGCTCATACTGATGTTTCTGCTTTGACC
TTCATCTTGCAATAATATGGTTCCAGGCTTGCAGTTGTTCTATGAAGGTAAATGGGTTACCGCT
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AtTT19

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CrCPR

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FaA3GT2

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MdF3H

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OsF3'H

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MsCHI

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PhAN9

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PhLDOX

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*Strep-tag-
PhLDOX*

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PtDFR

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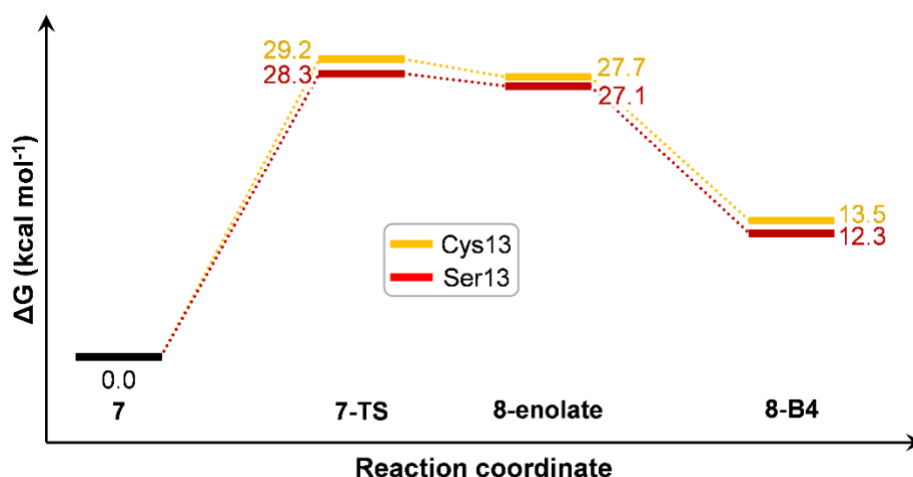
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<i>VvGST5</i>	ATGGCCGACGAAATTATCTTGTTGGATTTTTGGCCATCCATGTTTCGGTATGAGAGTTAGATTG GCTTTTGGCTGAAAAAGGTTTGAAGTACGAGTATAGAGAAGAGGACTTGTGGAACAAATCTCCC TTGTTGTTGGAAATGAACCCAGTTTCATAAGAAGATTCCAGCCTTGATTTCATAACGGTAAGCCA ATTTGCGAATCCTTGATTATCGTTTCAGTACATCGATGAAGTTTGGTGCGATAAGTCTCCATTA TTGCCATCTGATCCATACCAAAGAGCACAAAGCTAGATTTTGGGCTGATTACATCGATAAGAAG TTGTACGAACTGGGCAGAAAAATTTGGTCTACTAAGGGTGAAGAACAAAGAAACCGCTAAGAAA GAATTCATCGAGTGCTTGAAGTTGTTGGAGGGTGAATTGGGTGAAAAACCATATTTTGGTGGT GAGAAGATCGGTTTCGTTGATGTTGCTTTGGTTACTTTCTCTTGTGTTGTTCTACGCTTACGAA ACTTTTCGGTAACCTTCTCCATTGAAGCTGAATGCCCAAAATTTGATTGCTTGGACTAAGAGGTGC ATGGAAAAAGAATCTGTCTCCTCATCTTTGGAAGATCCACATAAGGTTTCATGGTTTCATCATG GGTATGAGAAAAGAGATTCGGTATCGAGTAG
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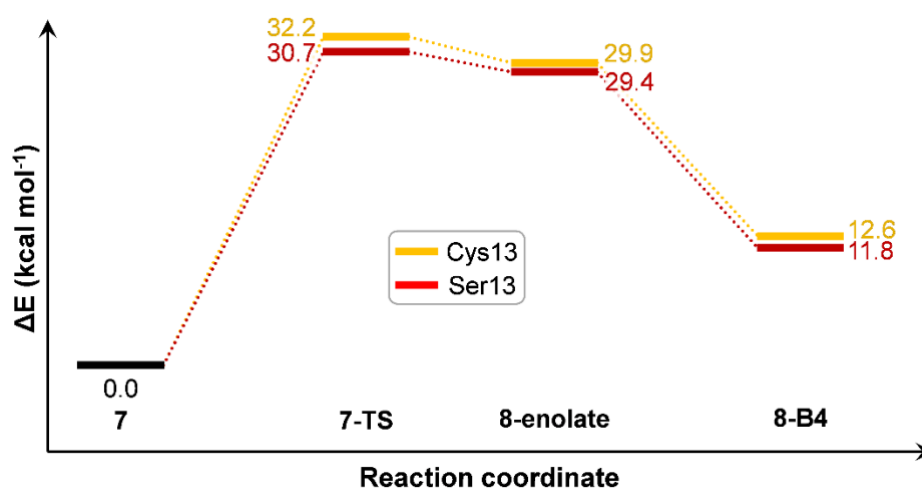
Supplementary Table 7. Individual datapoints for Fig. 3.

Variant	V_0^{GST} (%WT)			V_0^{DEH} (%WT)		
	Repl. 1	Repl. 2	Repl. 3	Repl. 1	Repl. 2	Repl. 3
V12G	25.2	24.5	22.7	23.5	25.3	24.6
V12W	101.5	93.7	92.8	5.3	6.0	4.3
V12Q	32.4	34.1	31.4	7.3	7.9	5.7
V12N	12.2	11.4	12.2	20.1	23.6	24.9
V12V	106.3	100.2	93.5	114.3	104.1	81.6
V12I	109.5	97.2	90.1	38.2	36.0	33.2
V12A	54.8	46.9	42.0	117.0	102.8	83.1
V12S	19.4	18.6	18.2	81.4	75.9	76.9
V12R	10.0	9.4	9.5	4.5	2.8	3.8
V12D	11.5	10.7	10.1	7.7	4.1	1.7
V12Y	13.6	10.8	11.0	5.8	4.3	4.5
V12L	63.9	61.5	61.0	15.8	13.7	18.7
V12M	66.8	59.0	58.8	6.8	4.1	6.0
V12F	40.5	35.2	34.3	6.2	4.7	6.4
V12E	9.1	11.5	8.4	6.0	4.1	4.0
V12C	28.9	23.5	24.4	92.7	81.0	74.2
V12T	17.4	16.3	17.9	124.1	104.6	106.9
V12P	13.1	12.0	11.2	114.5	105.2	94.4
C13R	9.9	8.6	9.3	4.4	2.1	2.5
C13V	10.1	8.8	9.7	4.4	1.9	2.7
C13W	9.7	8.1	8.5	3.2	2.3	2.9
C13A	160.3	156.6	158.2	50.8	46.4	39.9
C13K	10.1	8.6	9.5	4.4	4.2	3.4
C13L	5.8	9.0	7.2	4.0	3.0	4.2
C13P	10.4	10.6	10.3	4.4	3.8	5.3
C13G	149.0	139.3	139.5	76.4	64.5	61.6
C13C	103.6	97.0	99.5	113.9	103.3	82.8
C13Q	27.4	23.5	23.2	4.6	3.4	4.0
C13S	42.9	41.3	39.6	109.9	101.2	80.5
C13T	28.7	28.3	30.5	20.7	19.3	17.2
C13M	10.2	8.1	8.9	4.9	2.1	2.1
C13N	83.1	79.8	80.4	73.0	74.7	83.0
C13F	9.1	9.3	9.3	4.0	8.5	4.2
N108N	109.9	93.8	96.3	115.8	99.9	84.3
N108Q	41.5	36.1	36.6	31.5	26.9	38.3
N108G	50.6	47.7	50.8	53.2	48.6	48.1
N108A	46.2	42.6	41.0	11.9	11.3	14.1
N108K	12.1	11.2	10.5	4.3	2.7	4.1
N108S	32.6	28.5	27.0	11.2	11.1	14.0
N108H	63.9	59.4	57.0	8.4	5.5	8.1
N108R	13.0	12.6	12.5	4.7	4.1	4.3
N108W	39.0	38.7	39.1	4.3	3.1	3.3
N108E	31.7	24.7	27.6	21.1	17.4	21.6
N108V	10.3	8.4	8.9	3.3	2.7	2.9
N108C	32.3	26.2	26.8	14.3	13.8	17.7
N108Y	46.1	44.6	41.2	6.7	2.5	3.1
N108T	17.0	15.7	15.1	6.8	5.7	7.5
F112R	29.0	27.0	27.3	79.7	72.5	70.2
F112A	65.6	63.9	66.1	112.6	104.2	103.9
F112S	73.5	62.7	64.8	111.4	98.9	101.5

F112V	102.0	91.9	94.9	73.5	67.9	75.0
F112I	143.6	132.4	135.1	39.4	36.1	46.2
F112L	79.5	71.1	75.3	57.5	56.3	65.9
F112F	104.5	97.2	98.3	114.3	101.3	84.4
F112H	170.3	191.2	189.4	83.4	78.1	72.9
F112N	83.0	72.0	80.1	38.2	33.9	40.2
F112E	83.9	68.6	81.6	92.1	85.3	89.0
F112G	115.9	99.6	98.7	83.4	78.3	75.0
F112T	55.4	50.6	50.0	25.1	20.4	27.2
F112L	36.7	56.5	49.2	24.9	37.1	45.7
F112K	25.1	23.8	23.4	100.3	90.9	83.7
F112M	33.5	30.7	30.9	53.9	50.2	59.4
F112D	30.9	27.9	31.1	13.8	12.4	14.4
F112Y	47.6	41.7	44.1	147.1	133.8	113.1
F112W	50.1	43.1	46.2	118.9	103.7	98.6
F112Q	162.3	151.7	164.6	144.5	135.7	127.7
F112P	12.1	12.2	11.5	3.9	3.2	4.8
F112C	63.1	54.9	60.5	91.8	84.4	87.1



Supplementary Figure 1. Minimum free energy (ΔG) pathways calculated with ONIOM(ω B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P)//ONIOM(B3LYP/6-31G(d):ff14SB,gaff2,TIP3P) for the *PtGSTF8*-catalyzed reaction of flavan-3-on-4-ol ketone (**7**) into cyanidin (**8-B4**): wild-type enzyme (in yellow), C13S variant (in red). While QM/MM methods would ideally be supported with DFT/MM sampling methods when calculating energy profiles of variants with similar activities, we observed that the calculated free energies (ΔG) and potential energies (ΔE), to which no vibrational corrections was applied, were very similar for both investigated variants (Supplementary Figure 2) and thus plausibly capture the essence of the experimentally verified chemistry (Figure 3b).



Supplementary Figure 2. Minimum potential energy (ΔE) pathways calculated with ONIOM(ω B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P)//ONIOM(B3LYP/6-31G(d):ff14SB,gaff2,TIP3P) for the *PtGSTF8*-catalyzed reaction of flavan-3-on-4-ol ketone (**7**) into cyanidin (**8-B4**): wild-type enzyme (in yellow), C13S variant (in red).

Supplementary Table 8. Energies, entropies, and lowest frequencies of the lowest energy calculated structures.^a

Structure	E _{ONIOM} (Hartree) ^b	E _{ONIOM} + ZPE (Hartree) ^c	H (Hartree) ^d	S (cal mol ⁻¹ K ⁻¹) ^e	G (Hartree) ^f	Lowest freq. (cm ⁻¹)	# of imag. freq.
7 (WT)	-3052.416614	-3022.467263	-3020.698236	7309.2	-3024.171084	19.2	0
7-TS (WT)	-3052.365289	-3022.421828	-3020.653324	7305.9	-3024.124601	-257.3	1
8-enolate (WT)	-3052.368973	-3022.423014	-3020.653874	7309.7	-3024.126947	19.7	0
8-B4 (WT)	-3052.396607	-3022.446662	-3020.677840	7306.9	-3024.149606	19.4	0
7 (C13S)	-2729.441056	-2699.486986	-2697.718160	7309.0	-2701.190914	19.7	0
7-TS (C13S)	-2729.392161	-2699.443401	-2697.675367	7304.3	-2701.145853	-174.9	1
8-enolate (C13S)	-2729.394279	-2699.442552	-2697.673913	7311.2	-2701.147718	6.8	0
8-B4 (C13S)	-2729.422321	-2699.467380	-2697.698676	7308.7	-2701.171261	18.5	0
holo (WT)	-1946.897367	-1917.210779	-1915.458578	7250.6	-1918.903564	19.2	0
holo (C13S)	-1623.923333	-1594.231414	-1592.479479	7251.0	-1595.924642	17.4	0
7	-1105.356703	-1105.103731	-1105.084311	140.0	-1105.150844	22.0	0
8-B4	-1105.347400	-1105.094053	-1105.074481	139.1	-1105.140576	20.6	0

^a 1 Hartree = 627.51 kcal mol⁻¹. Thermal corrections at 298.15 K.

^b Calculated with ONIOM(ω B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P).

^c Calculated as $E_{\text{elec}}[\text{ONIOM}(\omega\text{B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P)}] + \text{ZPE}[\text{ONIOM}(\text{B3LYP/6-31G(d):ff14SB,gaff2,TIP3P})]$, where ZPE_{corr} is the zero-point energy.

^d Calculated as $E_{\text{elec}}[\text{ONIOM}(\omega\text{B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P)}] + H_{\text{corr}}[\text{ONIOM}(\text{B3LYP/6-31G(d):ff14SB,gaff2,TIP3P})]$, where H_{corr} is the thermal correction to enthalpy.

^e Calculated with ONIOM(B3LYP/6-31G(d):ff14SB,gaff2,TIP3P).

^f Calculated as $E_{\text{elec}}[\text{ONIOM}(\omega\text{B97X-D/6-311+G(2d,p):ff14SB,gaff2,TIP3P)}] + G_{\text{corr}}[\text{ONIOM}(\text{B3LYP/6-31G(d):ff14SB,gaff2,TIP3P})]$, where G_{corr} is the thermal correction to Gibbs free energy.