

Supplemental Material

Applied Microbiology and Biotechnology

Engineering yeast to enhance fraxetin production from ferulic acid and lignin

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Table S1 Codon-optimized sequences of gene used in this study

Gene	Encoding sequences
<i>COSY</i> from <i>Arabidopsis</i> <i>thaliana</i> (<i>AtCOSY</i>)	aggtctccaatggctactttggaatcactgacatcgcttggttcaaccatctaccaaccattgtc taacgacaaaactttgtctttgtcacttggacaacgacaacaacttgcacgtttcttcagata cttgagagtttactcttcttcttcttactgttgctgggaatctccatctgctgttcttcttctt ggctactgcttggttactactaccattggctgggttcttgagaagatctgcttctgacaacaga ttcgaattgttgttctgctgggtcaatctgtccattgggttaacgctactgttaactgtactttggaat ctgttggttacttggacgggtccagaccagggttctgtgaaagattgggtccagaccaactag agaagaaggatgggttaaccatgtatcttgcaagtactatgtccaatgtgggtgggtgggtttg gggtgcttctatccaccacgctatctgtgacgggttgggtgcttcttcttcaacgctatggctga attggctagagggtctactaagatctctatcgaaccagttgggacagagaaagattgttgggtc caagagaaaagccatgggttgggtgctccagttagagacttctgtcttggacaaggacttcgac ccatacgggcaagctatcgggtgacgttaagagagactgttcttctggtactgacgactcttggga ccaattgaaggctcaattgttggaaaagtctggttgaacttcactacttctgaagcttgggtgct tacatctggagagctaagggttagagctgctaagactgaagaagagaaacgttaagttcgttact ctatcaacatcagaagattgatgaaccaccattgccaagggttactggggttaacgggtgtgtc caatgtacgctcaaatcaaggctgggtgaattgatcgaacaaccaatctggaagactgctgaatt gatcaagcaatctaagtctaacttctgacgaatacgttagatcttcatcgactccaagaattg caccacaaggacgggtatcaacgctgggtactgggtatgttacatcttgagaatctgttcggtaaca gatggaaactaagggttaagggttcttctgtatcttcggttcttgggtgggtatcggttaaaggagacct
<i>S8H</i> from <i>Arabidopsis</i> <i>thaliana</i> (<i>AtS8H</i>)	aggtctccaatgggtattaatttcgaagatcaaacctacattgtttaatttcgttgtagagagggtga acgggtgtaagggtatgattgattctggttcttcagttccaagaccattttgttcaaccattatca gaaagaattccaactcaaaaagcttctgacttgaagcaacacaaccaattgattgtctaatttg gatgggtccacaacataaggaagttgcaaagcaaatcgttgaagctgcagaaacattgggttct ttcaagttgtaatatcatgggtgttctcagttgaattgttgaattgttgaatcttcagctcatgaattttc ctcaagcaccagaagaaaaatctatgtattgaaggaagtttctccatcaaagttgggttaagtacg gtacttcattcgttccagataaggaaaaggctatcgaatggaaggattacgttctatgtgtaca caaacgattcagaagctttacaacattggccacaacctgtagagaagttgcattggaatttttaa attcttcaatggaaatgggttaaaaatgttgttaacatcttgatggaaaacgttgggtgttactttgga gaagaaaagatgaacgggttgggtgacaaagatgggttaacatgaactactaccaactgtc catctccagaattaacagttgggtgttggtagacattcagatatgggcatgttgactgtttgttaca agatgggtatcgggtgttgtacgttaagttggataacgggtgaatgggctgaaattccaccagttc atgggtgcattagttattaatatcgggtgacactttgcaaattcttctaacgggtaaatacaaatcagc tgaacatagagttagaactacaacatcgggtctagagttcagttccaatttttacagcaccaaat ccatctcaaaaagttgggtccattaccagaagttgttaaaaagagatgggtgtgctagatacaagga attcttgtccaagattacatgaataatttcttgggtcaaccacatgatggtaaaaagcttggatttt gctagagcagaataaaggagacct

Table S2 Plasmids used in this study

Name	Description	Source
pScop-7	ZCH415_ <i>T_{CYC1}</i> -P _{<i>TEF1</i>} -T _{<i>PDC1</i>}	(Zhao et al. 2020)
pScop-15	ZCH415_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtF6'H1</i> -T _{<i>CYC1</i>}	(Zhao et al. 2020)
pScop-16	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>Pc4CL</i> -T _{<i>CYC1</i>}	(Zhao et al. 2020)
pScop-38	ZCH415_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} -T _{<i>CYC1</i>}	(Zhao et al. 2020)
pScop-39	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} -T _{<i>CYC1</i>}	(Zhao et al. 2020)
pUC57	Plasmid used to harbor the synthesized <i>AtCOSY</i> gene	SynbioB (Tianjin, China)
pUC19	Plasmid used to harbor the synthesized <i>AtS8H</i> gene	Genscript (Nanjing, China)
pRS426	Plasmid used to overexpress gene (high copy plasmid)	(Zhou et al. 2021)
pHBT089	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT091	ZCH415_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -T _{<i>CYC1</i>}	This study
pHBT093	ZCH415_ <i>T_{CYC1}</i> -P _{<i>TEF1</i>} - <i>AtCOSY</i> -T _{<i>PDC1</i>}	This study
pHBT105	pRS426_P _{<i>TDH3</i>} - <i>Pc4CL</i> -T _{<i>CYC1</i>}	This study
pHBT106	pRS426_P _{<i>TDH3</i>} - <i>AtF6'H1</i> -T _{<i>CYC1</i>}	This study
pHBT107	pRS426_P _{<i>TDH3</i>} - <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT108	pRS426_P _{<i>TDH3</i>} - <i>AtCOSY</i> -T _{<i>CYC1</i>}	This study
pHBT111	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -GSG- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT112	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -GSGEAAAK- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT113	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -GS2- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT114	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -GS4- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT115	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -(GGGGS)1- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT116	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -(GGGGS)2- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study
pHBT117	ZCH416_ <i>T_{TDH3}</i> -P _{<i>TDH3</i>} - <i>AtCOSY</i> -(GGGGS)3- <i>AtS8H</i> -T _{<i>CYC1</i>}	This study

pHBT118	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-GSG-AtCOSY-T _{CYC1}	This study
pHBT119	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H- GSGEAAAK- AtCOSY-T _{CYC1}	This study
pHBT120	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-GS2-AtCOSY-T _{CYC1}	This study
pHBT121	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-GS4-AtCOSY-T _{CYC1}	This study
pHBT122	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-(GGGGS)1-AtCOSY- T _{CYC1}	This study
pHBT123	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-(GGGGS)2-AtCOSY- T _{CYC1}	This study
pHBT124	ZCH416_T _{TDH3} -P _{TDH3} -AtS8H-(GGGGS)3-AtCOSY- T _{CYC1}	This study

Table S3 Primers used in this study

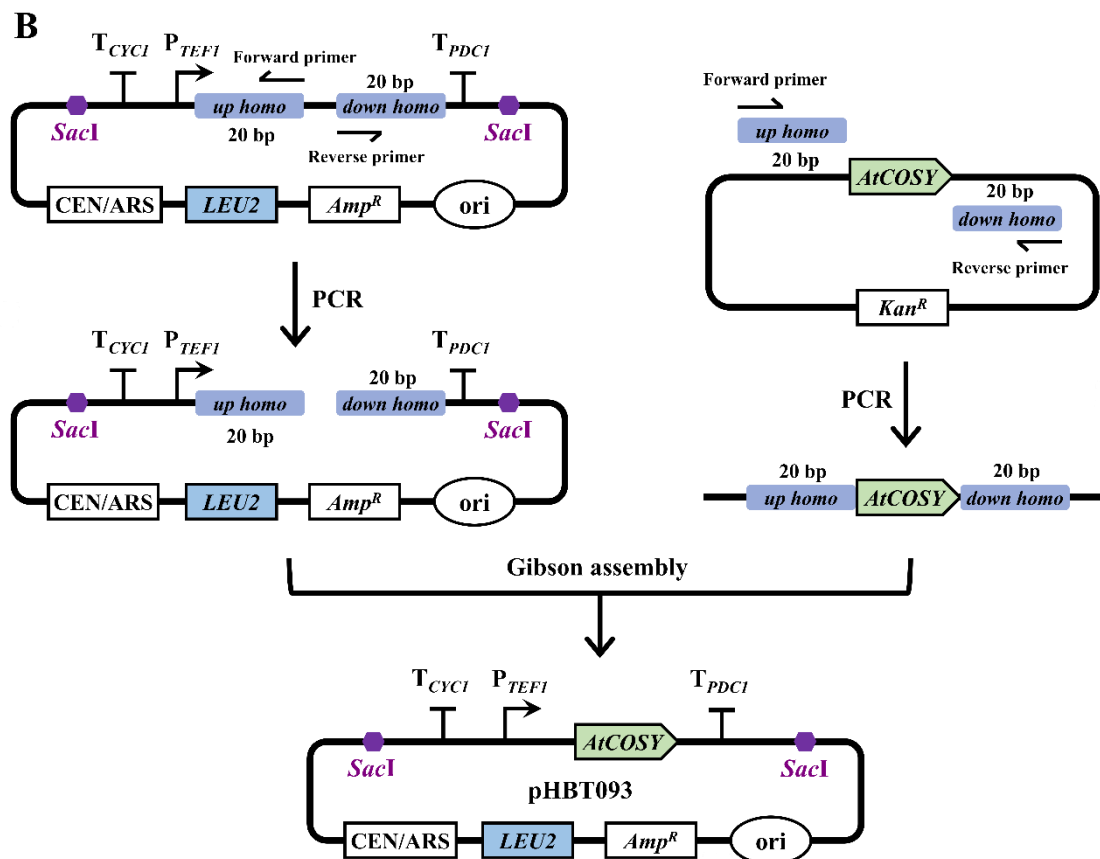
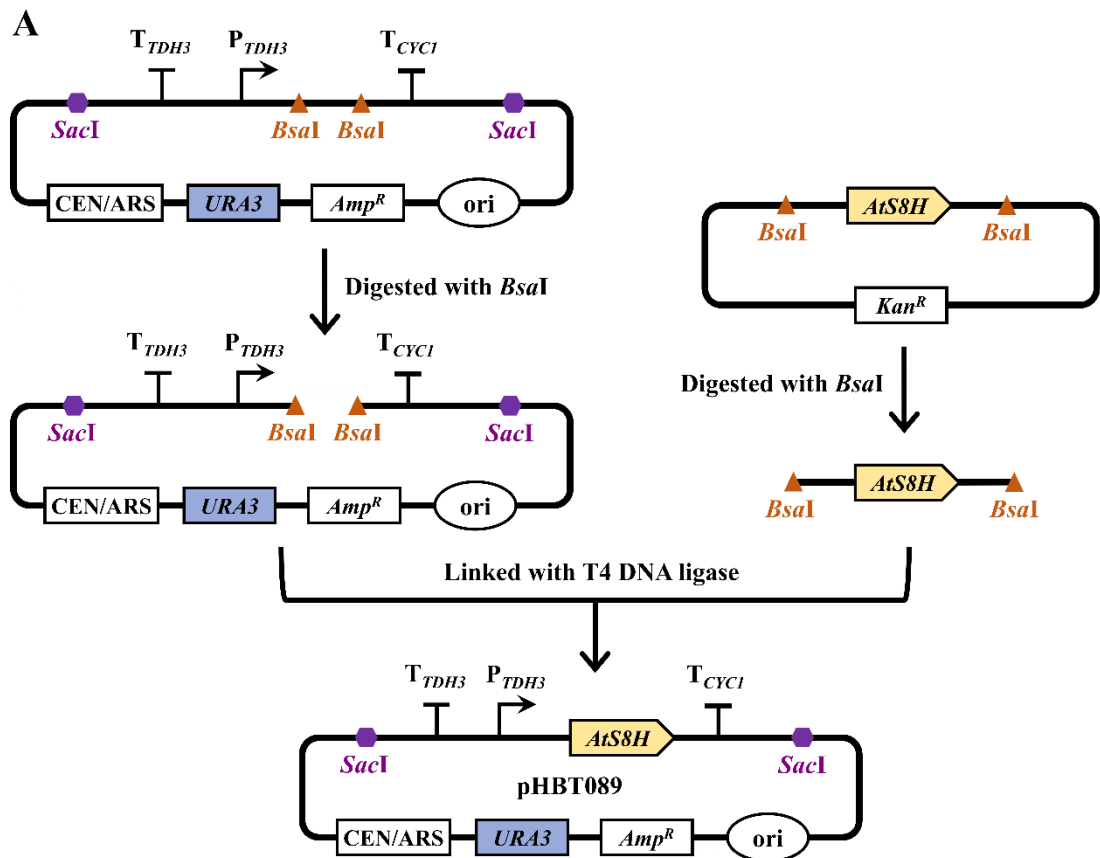
Primer	Sequences
YZ-S8H-089-S-F	CTGCCTGGAGTAAATGATGACAC
YZ-S8H-089-S-R	CTTTGCAACTTCCTTATGTTGTGGA
YZ-S8H-089-X-F	GAGTTAGAACTACAAACATCGGTTCTAG
YZ-S8H-089-X-R	GATAACAATTCACACAGGAAACAGC
COSY-HP-063-F	CCTAAAGCGATTTAATCTCTAATTATT
COSY-HP-063-R	CTCCCATTTTGTAAATAAAACCTTAGATT
COSY-093-F	TTTAAATTACAAAATGGGAGATGGCTACTTTGGAAAT CACTGAC
COSY-093-R	AGAGATTAAATCGCTTTAGGTAAACGATACCAACC AAGAAACCGAA
YZ-COSY-093-S-F	ACCGCCTCGTTTCTTTTCTTC
YZ-COSY-093-S-R	CCAGCAGAACACAACAATTCGA
YZ-COSY-093-X-F	TCCAATGTACGCTCAAATCAAGG
YZ-COSY-093-X-R	TGGTTCCACTAATTCATCGGTTTTT
Leu-F	TGATAATAATATTTATAGAATTGTGTAGAATTGCAGAT TCGGGAAATGGTTCAAGAAGGTATTGA
Leu-R	AACTGTGGGAATACTCAGGTAT
YORWdelta17-LH-F	GATTTCAAGCGGTAAATACGAAG
YORWdelta17-LH-R	GAATCTGCAATTCTACACAATTCT
LH-S8H-RH-F	TGATAATAATATTTATAGAATTGTGTAGAATTGCAGAT TCAGTTTATCATTATCAATACTGCCATTT
LH-S8H-RH-F	ATAATATTAGGTATGTAGAATGTACTAGAAGTTCTCC TCGGAACAAAAGCTGGAGCTCAAAG
YORWdelta17-RH-F	CGAGGAGAACTTCTAGTACATTC
YORWdelta17-RH-R	TTAAGAAGTGCAATCTAAGGCG
LH-COSY-RH-F	TGATAATAATATTTATAGAATTGTGTAGAATTGCAGAT TCTACATATCACATAGGAAGCAACAGG
LH-COSY-RH-R	ATAATATTAGGTATGTAGAATGTACTAGAAGTTCTCC TCGGCAGTTTTGAATTGAGTAACCAT
LH-S8H-COSY-F	TGATAATAATATTTATAGAATTGTGTAGAATTGCAGAT TCAGTTTATCATTATCAATACTGCCATTT
LH-S8H-COSY-R	GAACAAAAGCTGGAGCTCAA
S8H-COSY-RH-F	TTTTGGGACGCTCGAAGGCTTTGAGCTCCAGCTTTT GTTCTACATATCACATAGGAAGCAACAGG
S8H-COSY-RH-R	ATAATATTAGGTATGTAGAATGTACTAGAAGTTCTCC TCGGCAGTTTTGAATTGAGTAACCAT
TDH3p-GBDgene- CYC1t-F	CGAGGTCGACGGTATCGATAATCTAGATGCAGTAATA TACACAGATTCC
TDH3p-GBDgene- CYC1t-R	GCTGCAGGAATTCGATATCAAAAGCCTTCGAGCGTC CCAAAA
LH-Ura-F	CTCCCCAAGAATATAGGAATCC

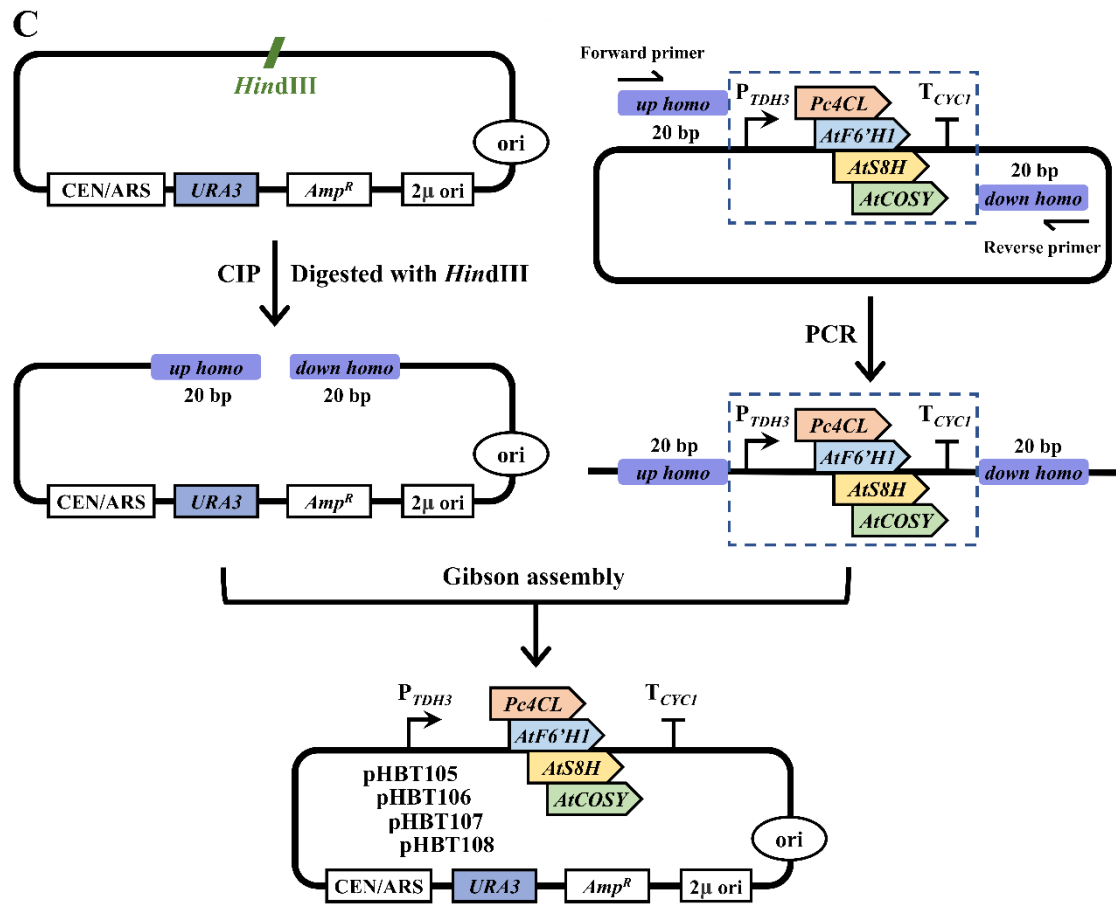
LH-Ura-R	AAAAAAAAAATGATGAATTGAATTGAAAAGCTGTGG TATGGCTTCCTTCCCGTTATACTTTGT
Ura-F	CCATACCACAGCTTTTCAATTCA
Ura-R	GTTCCGATTTAGTGCTTTACG
Ura-S8H-LH-F	TTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCG GAACAGTTTATCATTATCAATACTGCCATTT
Ura-S8H-LH-R	GTAATTTGATAATTGTTTCGGATTGCATTGTTGCCGAA TCCGAACAAAAGCTGGAGCTCAAAG
Ura-COSY-LH-F	TTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCG GAACTACATATCACATAGGAAGCAACAGG
Ura-COSY-LH-R	GTAATTTGATAATTGTTTCGGATTGCATTGTTGCCGAA TCCGCAGTTTTGAATTGAGTAACCATT
SHC-LH-F	GGATTCTGGCAACAATGCAA
SHC-LH-R	GCAGTGTAAGAAGGTGTACTG
LH-His-F	CTACAATGCATATTGGGAGGT
LH-His-R	TTACCGAGGCATAAAAAAATATAGAGTGTACTAGAG GAGGCCTCAAAATGGAATCCATATTTCTG
His-F	CCTCCTCTAGTACACTCTATATTTTTT
His-R	TACAATTTCTGATGCGGTATTTT
His-COSY-LH-F	ACAGATGCGTAAGGAGAAAATACCGCATCAGGAAA TTGTATACATATCACATAGGAAGCAACAGG
His-COSY-LH-R	AAACCATTAAATCTTCTTCTTTGATGGTGAGAATTGG GTGGCAGTTTTGAATTGAGTAACCATT
COSY-RH-F	CACCCAATTCTCACCATCAAA
COSY-RH-R	TGCAACATTGGCAGATAAATCT
YZ-150-S-F	CTTCTATTACCTTCTGCTCTCTCTGAT
YZ-150-S-R	TTCCAAGAATGGAACGATATCGAAC
YZ-150-X-F	GAGAGCTGGTGTAACCTATCTTGATC
YZ-150-X-R	CAAAACCTTCTCAAGCAAGGTTTT
YZ-151-S-F	CTTCTATTACCTTCTGCTCTCTCTGAT
YZ-151-S-R	GCATTGCGTCACCGATATTAATAAC
YZ-151-X-F	GGTGTTCCATTGGAAGTTTTAGATGA
YZ-151-X-R	CAAAACCTTCTCAAGCAAGGTTTT
YZ-152-S-F	CTTCTATTACCTTCTGCTCTCTCTGAT
YZ-152-S-R	TAATAACTAATGCACCATGAACTGGTG
YZ-152-X-F	GTTAATCATGGTGTTTCAGTTGAATTGT
YZ-152-X-R	CAAAACCTTCTCAAGCAAGGTTTT
YZ-153-S-F	CTTCTATTACCTTCTGCTCTCTCTGAT
YZ-153-S-R	GCTCTAACCTTAGCTCTCCAGATGTAA
YZ-153-X-F	CCCAACTAGAGAAGAAGGTATGGT
YZ-153-X-R	CAAAACCTTCTCAAGCAAGGTTTT
CX-Linker-F	TTTGGACCAATTGAAGGCTCAAT
CX-Linker-R	AACGTTGGTGTTACTTTGGAAGAA

Table S4 Strains used in this study

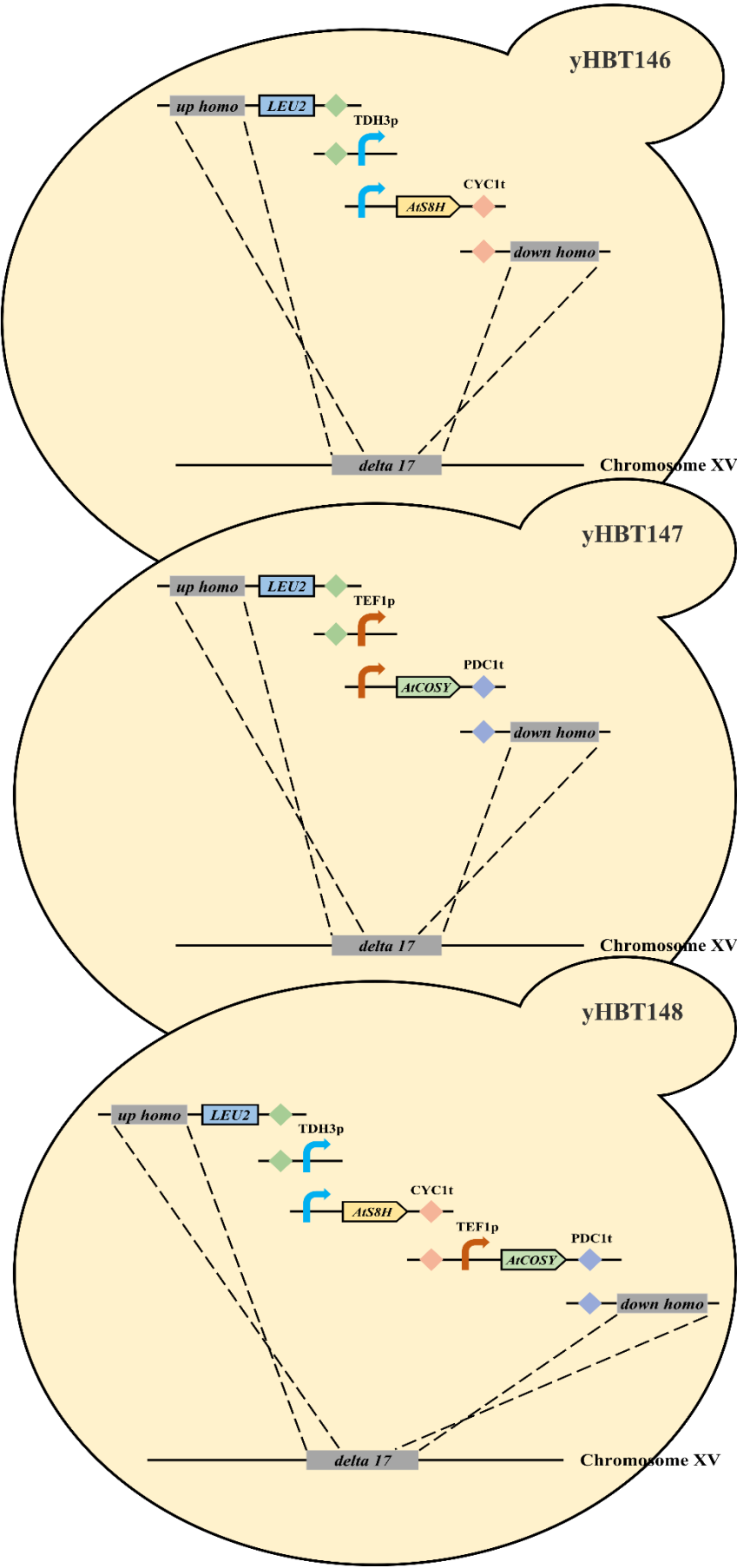
Yeast Strains	Description	Source
BY4742	<i>MATα</i> , <i>his3</i> Δ 1, <i>leu2</i> Δ 0, <i>lys2</i> Δ 0, <i>ura3</i> Δ 0	https://www.yeastgenome.org/strain/by4742
yZCH21	BY4742, <i>ho::his3_T_{TDH3}-P_{TDH3}-AtF6'H1-(GGGGS)₄-Pc4CL-T_{CYC1}</i>	(Zhao et al. 2020)
yHBT122	yZCH21, pScop-39 (ZCH416_T _{TDH3} -P _{TDH3} -T _{CYC1}), pScop-38 (ZCH415_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study
yHBT137	yZCH21, pHBT089 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtS8H</i> -T _{CYC1})	This study
yHBT138	yZCH21, pScop-39 (ZCH416_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study
yHBT139	yZCH21, pHBT093 (ZCH415_T _{CYC1} -P _{TEF1} - <i>AtCOSY</i> -T _{PDC1})	This study
yHBT140	yZCH21, pScop-7 (ZCH415_T _{CYC1} -P _{TEF1} -T _{PDC1})	This study
yHBT141	yZCH21, pHBT089 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtS8H</i> -T _{CYC1}), pHBT093 (ZCH415_T _{CYC1} -P _{TEF1} - <i>AtCOSY</i> -T _{PDC1})	This study
yHBT142	yZCH21, pScop-39 (ZCH416_T _{TDH3} -P _{TDH3} -T _{CYC1}), pScop-7 (ZCH415_T _{CYC1} -P _{TEF1} -T _{PDC1})	This study
yHBT146	yZCH21, <i>YORWdelta17::leu2_P_{TDH3}-AtS8H</i> -T _{CYC1}	This study
yHBT147	yZCH21, <i>YORWdelta17::leu2_P_{TEF1}-AtCOSY</i> -T _{PDC1}	This study
yHBT148	yZCH21, <i>YORWdelta17::leu2_P_{TDH3}-AtS8H</i> -T _{CYC1} -P _{TEF1} - <i>AtCOSY</i> -T _{PDC1}	This study
yHBT180	yZCH21, pHBT089 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtS8H</i> -T _{CYC1}), pHBT091 (ZCH415_T _{TDH3} -P _{TDH3} - <i>AtCOSY</i> -T _{CYC1})	This study
yHBT181	yZCH21, pHBT111 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtCOSY</i> -GSG- <i>AtS8H</i> -T _{CYC1}), pScop-38 (ZCH415_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study
yHBT182	yZCH21, pHBT112 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtCOSY</i> -GSGEAAAK- <i>AtS8H</i> -T _{CYC1}), pScop-38 (ZCH415_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study
yHBT183	yZCH21, pHBT113 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtCOSY</i> -GS2- <i>AtS8H</i> -T _{CYC1}), pScop-38 (ZCH415_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study
yHBT184	yZCH21, pHBT114 (ZCH416_T _{TDH3} -P _{TDH3} - <i>AtCOSY</i> -GS4- <i>AtS8H</i> -T _{CYC1}), pScop-38 (ZCH415_T _{TDH3} -P _{TDH3} -T _{CYC1})	This study

yHBT185	yZCH21, pHBT115 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtCOSY</i> -(GGGGS)1- <i>AtS8H</i> - T_{CYC1}), pScop-38	This study
yHBT186	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT116 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtCOSY</i> -(GGGGS)2- <i>AtS8H</i> - T_{CYC1}), pScop-38	This study
yHBT187	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT117 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtCOSY</i> -(GGGGS)3- <i>AtS8H</i> - T_{CYC1}), pScop-38	This study
yHBT188	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT118 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -GSG- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT189	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT119 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> - GSGEAAAK- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT190	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT120 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -GS2- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT191	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT121 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -GS4- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT192	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT122 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -(GGGGS)1- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT193	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT123 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -(GGGGS)2- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT194	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yZCH21, pHBT124 (ZCH416_ T_{TDH3} - P_{TDH3} - <i>AtS8H</i> -(GGGGS)3- <i>AtCOSY</i> - T_{CYC1}), pScop-38	This study
yHBT195	(ZCH415_ T_{TDH3} - P_{TDH3} - T_{CYC1}) yHBT148, <i>YERCdelta8::Ura3</i> _ P_{TDH3} - <i>AtS8H</i> - T_{CYC1}	This study
yHBT196	yHBT148, <i>YERCdelta8::Ura3</i> _ P_{TEF1} - <i>AtCOSY</i> - T_{PDC1}	This study
yHBT198	yHBT195, <i>YOLCdelta1::His3</i> _ P_{TEF1} - <i>AtCOSY</i> - T_{PDC1}	This study





D



E

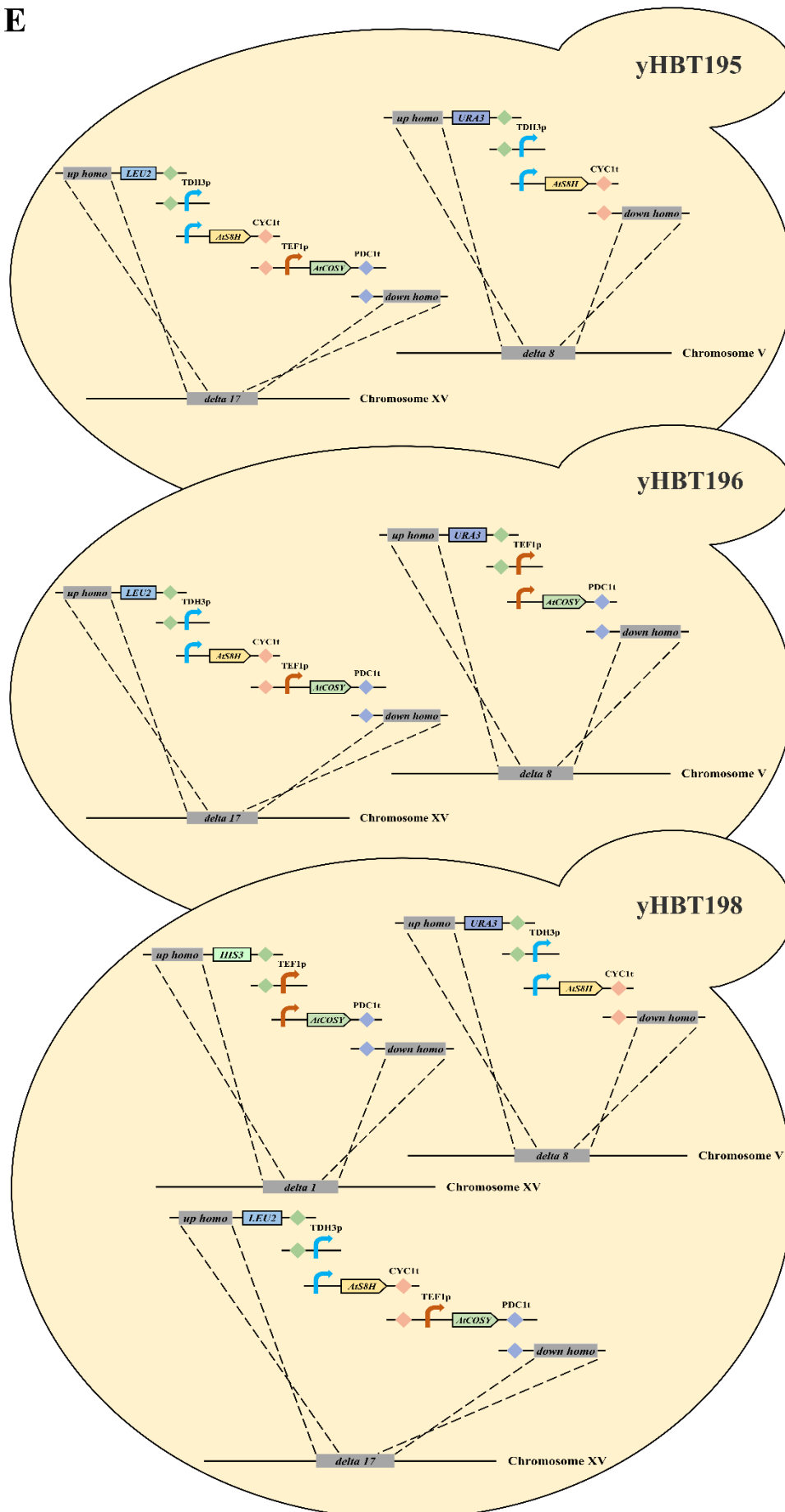


Fig. S1 Flow chart of the plasmid construction and genome integration. (A) Flow chart of *AtS8H* plasmid pHBT089. (B) Flow chart of *AtCOSY* plasmid pHBT093. (C) Flow chart of genome integration. (D) Flow chart of the overexpression plasmid pHBT105, pHBT106, pHBT107, pHBT108. (E) The flow chart shows the increase in copy number on the genome. The black arrows represent either digestion, ligase attachment, or assembly. The curved arrows in different colors represent different promoters. The diamonds in different colors represent different terminators. The rectangles in different colors represent different Nutritional deficiency labels. The gray rectangles on the left of nutrient deficiency labels or the right of terminators represent upstream or downstream homologous arms. The gray rectangles on the chromosome represent different *delta* sites.

Reference

- Zhao CH, Zhang RK, Qiao B, Li BZ, Yuan YJ (2020) Engineering budding yeast for the production of coumarins from lignin. *Biochem Eng J* 160: 107634. <https://doi.org/10.1016/j.bej.2020.107634>
- Zhou MY, Bi YH, Ding MZ, Yuan YJ (2021) One-step biosynthesis of vitamin C in *Saccharomyces cerevisiae*. *Front Microbiol* 12: 643472. <https://doi.org/10.3389/fmicb.2021.643472>