Supporting information

Exploring Catalysis Specificity of Phytoene Dehydrogenase CrtI in Carotenoid Synthesis

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Supplementary Table S1. The percentage of dehydrogenation products in *Bt*CrtI and mutants of error-prone PCR and mutation site verification

	Lycopene [a]	Neurosporene [a]	ζ-Carotene [a]	Phytofluene [a]	Ratio [b]
WT	80.3%	17.1%	1.3%	1.3%	66.4%
H136R&Y160F	37.7%	39.1%	13.4%	9.8%	45.8%
H453R&N576S	0.0%	3.1%	59.9%	37.0%	15.2%
H136R	46.4%	29.1%	12.5%	11.9%	42.2%
Y160F	78.3%	18.9%	1.6%	1.3%	67.2%
H453R	0.0%	12.6%	51.8%	35.6%	14.5%
N576S	79.7%	17.5%	1.5%	1.4%	66.5%
H136R&H453R	0.0%	0.0%	0.0%	100.0%	0.2%
H136R&N576S	59.2%	23.7%	10.1%	7.1%	55.2%
Y160F&H453R	0.0%	14.6%	55.5%	29.9%	22.8%
Y160F&N576S	82.7%	15.3%	1.1%	1.0%	74.9%
H136R&Y160F&H453R	0.0%	0.0%	0.0%	100.0%	0.3%
H136R&Y160F&N576S	50.7%	30.6%	11.3%	7.4%	57.8%
H136R&H453R&N576S	0.0%	0.0%	0.0%	100.0%	0.3%
Y160F&H453R&N576S	0.0%	14.0%	55.9%	30.1%	22.6%
H136R&Y160F&H453R&N576S	0.0%	0.0%	0.0%	100.0%	0.4%

[a] Lycopene, neurosporene, ζ-carotene, and phytofluene represented the proportion of themselves in total dehydrogenation products, respectively.

[b] Ratio represented the proportion of dehydrogenation products in total carotenoids.

Supplementary Table S2. The percentage of dehydrogenation products in saturated mutants of *Bt*CrtI at H136 and H453 sites

	Lycopene [a]	Neurosporene [a]	ζ-Carotene [a]	Phytofluene [a]	Ratio [b]
H136A	81.2%	12.9%	4.1%	1.8%	68.2%
H136P	19.8%	20.4%	24.9%	34.9%	13.0%
H136C	83.9%	11.9%	2.9%	1.3%	73.4%
H136G	60.7%	21.6%	9.5%	8.1%	45.5%
H136S	81.8%	13.2%	3.4%	1.6%	70.6%
H136K	77.0%	19.2%	2.0%	1.8%	65.8%
H136Q	75.3%	20.0%	3.0%	1.7%	70.1%
H136F	77.4%	19.7%	1.6%	1.2%	70.1%
H136E	68.1%	23.1%	4.8%	4.1%	57.3%
H136Y	78.1%	18.7%	2.0%	1.2%	69.4%
H453A	73.3%	23.3%	1.9%	1.5%	70.5%
H453P	66.3%	22.6%	5.1%	5.9%	59.7%
H453C	49.1%	36.9%	8.1%	6.0%	50.3%
H453G	65.4%	30.4%	2.4%	1.8%	74.3%
H453S	55.3%	31.4%	7.5%	5.8%	55.6%
H453K	0.5%	20.7%	54.8%	24.0%	16.7%
H453Q	44.7%	46.1%	5.0%	4.3%	51.2%
H453F	65.9%	30.0%	2.6%	1.4%	70.3%
H453E	59.6%	34.1%	3.2%	3.1%	65.2%
H453Y	41.4%	45.5%	7.0%	6.0%	40.8%

[a] Lycopene, neurosporene, ζ-carotene, and phytofluene represented the proportion of themselves in total dehydrogenation products, respectively.

[b] Ratio represented the proportion of dehydrogenation products in total carotenoids.

Supplementary Table S3. The percentage of dehydrogenation products in combined mutants of *Bt*CrtI at H136 and H453 sites

	Lycopene [a]	Neurosporene [a]	ζ-Carotene [a]	Phytofluene [a]	Ratio [b]
H136C&H453G	68.1%	16.1%	8.4%	7.5%	63.9%
H136C&H453F	61.1%	14.1%	16.7%	8.1%	61.2%
H136C&H453E	47.8%	14.4%	15.9%	21.9%	36.1%
H136S&H453G	55.8%	16.5%	13.9%	13.8%	52.7%
H136S&H453F	31.7%	11.0%	29.1%	28.2%	33.7%
H136S&H453E	8.8%	9.7%	20.4%	61.2%	11.0%
H136P&H453G	0.0%	0.0%	0.0%	100.0%	3.5%
H136P&H453F	0.0%	0.0%	0.0%	100.0%	1.2%
H136P&H453E	0.0%	0.0%	0.0%	100.0%	0.2%
H136C&H453R	0.0%	0.0%	0.0%	100.0%	0.3%
H136S&H453R	0.0%	0.0%	0.0%	100.0%	0.1%
H136P&H453R	0.0%	0.0%	0.0%	100.0%	0.1%

[a] Lycopene, neurosporene, ζ-carotene, and phytofluene represented the proportion of themselves in total dehydrogenation products, respectively.

[b] Ratio represented the proportion of dehydrogenation products in total carotenoids.

Supplementary Table S4. Primers used in this study

Primers	Sequence（5'- 3'）
For construction of GAL1p-*Pa*CrtB-PGK1t, *TRP1* homologous arm	
TRP1-LF[a]	*GTTTAAAC*GGAAGAGGAGTAGGGAA
TRP1-LR	TACGATGCTGTTCTATTAAATGCT
GAL1p-F[b]	AGCATTTAATAGAACAGCATCGTATTATATTGAATTTTCAAAA ATTCTTAC
GAL1p-R	TATAGTTTTTTCTCCTTGACGTTAAAG
PaCrtB-F[b]	GTCAAGGAGAAAAAACTATAATGTCACAACCACCATTATTGG
PaCrtB-R[b]	CTATCGATTTCAATTCAATTCAATTTAAACAGGTCTTTGCCAT AAACC
PGK1t-F	ATTGAATTGAATTGAAATCGATAG
PGK1t-R[b]	CGTCATAACTGCAAAGTACACATATATAACGAACGCAGAATT TTCGAG
TRP1-RF	ATATATGTGTACTTTGCAGTTATGACG
TRP1-RR[a]	*GTTTAAAC*ACGCCAACCAAGTATTT
For construction of GAL7p-*Bt*CrtI-CYC1t	
GAL7p-F[a]	*TCTAGA*TTTGCCAGCTTACTATCCTTCTTG
GAL7p-R[b]	GCTTCTTCTGATCAGACATTTTTGAGGGAATATTCAACTG
BtCrtI-F	ATGTCTGATCAGAAGAAGCAC
BtCrtI-R	TTATATCCTAATATCGTTAGAGTTCTGTCC
CYC1t-F[b]	CTAACGATATTAGGATATAAGGCCGCATCATGTAATTAGTTAT G
CYC1t-R[a]	*GCGGCCGC*GCAAATTAAAGCCTTCGAG
For error-prone PCR	
LHA-GAL7p-F	TCGAGGTCGACGGTATCGATAAGCT
BtCrtI-R-1	CGACAATGTGCTTCTTCTGATCAGACAT
BtCrtI-F-1	ATTCCCTCAAAAATGTCTGATCAGAAGAAGCACATTGTCG
BtCrtI-R-2	TAATTACATGATGCGGCCTTATATCCTAATATCGTTAGAG
CYC1t-F-1	CTCTAACGATATTAGGATATAAGGCCGCATC
RHA-CYC1t-R	TGATTACGCCAAGCGCGCAATTAACC
For construction of GAL7p-*Bt*CrtI-Histag-CYC1t	
GAL7p-F[a]	*TCTAGA*TTTGCCAGCTTACTATCCTTCTTG
*Bt*CrtI-Histag-	ACTAATTACATGATGCGGCCTTAATGATGATGATGATGATGTA

R[b]	TCCTAATATCGTTAGAGTTCTG
CYC1t-F-2	GGCCGCATCATGTAATTAGTTATG
CYC1t-R[a]	*GCGGCCGC*GCAAATTAAAGCCTTCGAG

[a] Restriction site was in italic.

[b] Homologous sequence was underlined.

Supplementary Table S5. Plasmids used in this study

Plasmid	Description	Source
pCC01	pEASY-Blunt Zero possessing *TRP1* homologous arm with *TRP1* marker, GAL1p-*Pa*CrtB-PGK1t	This study
pCC02	pRS416-GAL7p-*Bt*CrtI-CYC1t	This study
pCC03	pRS416-GAL7p-*Bt*CrtI(H136R&Y160F)-CYC1t	This study
pCC04	pRS416-GAL7p-*Bt*CrtI(H453R&N576S)-CYC1t	This study
pCC05	pRS416-GAL7p-*Bt*CrtI(H136R)-CYC1t	This study
pCC06	pRS416-GAL7p-*Bt*CrtI(Y160F)-CYC1t	This study
pCC07	pRS416-GAL7p-*Bt*CrtI(H453R)-CYC1t	This study
pCC08	pRS416-GAL7p-*Bt*CrtI(N576S)-CYC1t	This study
pCC09	pRS416-GAL7p-*Bt*CrtI_WT-Histag-CYC1t	This study
pCC10	pRS416-GAL7p-*Bt*CrtI(H136R)-Histag-CYC1t	This study
pCC11	pRS416-GAL7p-*Bt*CrtI(H453R)-Histag-CYC1t	This study
pCC12	pRS416-GAL7p-*Bt*CrtI(H136A)-CYC1t	This study
pCC13	pRS416-GAL7p-*Bt*CrtI(H136P)-CYC1t	This study
pCC14	pRS416-GAL7p-*Bt*CrtI(H136C)-CYC1t	This study
pCC15	pRS416-GAL7p-*Bt*CrtI(H136G)-CYC1t	This study
pCC16	pRS416-GAL7p-*Bt*CrtI(H136S)-CYC1t	This study
pCC17	pRS416-GAL7p-*Bt*CrtI(H136K)-CYC1t	This study
pCC18	pRS416-GAL7p-*Bt*CrtI(H136Q)-CYC1t	This study
pCC19	pRS416-GAL7p-*Bt*CrtI(H136F)-CYC1t	This study
pCC20	pRS416-GAL7p-*Bt*CrtI(H136E)-CYC1t	This study
pCC21	pRS416-GAL7p-*Bt*CrtI(H136Y)-CYC1t	This study
pCC22	pRS416-GAL7p-*Bt*CrtI(H453A)-CYC1t	This study
pCC23	pRS416-GAL7p-*Bt*CrtI(H453P)-CYC1t	This study
pCC24	pRS416-GAL7p-*Bt*CrtI(H453C)-CYC1t	This study
pCC25	pRS416-GAL7p-*Bt*CrtI(H453G)-CYC1t	This study
pCC26	pRS416-GAL7p-*Bt*CrtI(H453S)-CYC1t	This study
pCC27	pRS416-GAL7p-*Bt*CrtI(H453K)-CYC1t	This study
pCC28	pRS416-GAL7p-*Bt*CrtI(H453Q)-CYC1t	This study
pCC29	pRS416-GAL7p-*Bt*CrtI(H453F)-CYC1t	This study
pCC30	pRS416-GAL7p-*Bt*CrtI(H453E)-CYC1t	This study
pCC31	pRS416-GAL7p-*Bt*CrtI(H453Y)-CYC1t	This study
pCC32	pRS416-GAL7p-*Bt*CrtI(H136C&H453G)-CYC1t	This study
pCC33	pRS416-GAL7p-*Bt*CrtI(H136C&H453F)-CYC1t	This study
pCC34	pRS416-GAL7p-*Bt*CrtI(H136C&H453E)-CYC1t	This study
pCC35	pRS416-GAL7p-*Bt*CrtI(H136S&H453G)-CYC1t	This study
pCC36	pRS416-GAL7p-*Bt*CrtI(H136S&H453F)-CYC1t	This study
pCC37	pRS416-GAL7p-*Bt*CrtI(H136S&H453E)-CYC1t	This study
pCC38	pRS416-GAL7p-*Bt*CrtI(H136P&H453G)-CYC1t	This study
pCC39	pRS416-GAL7p-*Bt*CrtI(H136P&H453F)-CYC1t	This study
pCC40	pRS416-GAL7p-*Bt*CrtI(H136P&H453E)-CYC1t	This study

pCC41	pRS416-GAL7p-*Bt*CrtI(H136C&H453R)-CYC1t	This study
pCC42	pRS416-GAL7p-*Bt*CrtI(H136S&H453R)-CYC1t	This study
pCC43	pRS416-GAL7p-*Bt*CrtI(H136P&H453R)-CYC1t	This study
pCC82	pRS416-GAL7p-*Bt*CrtI(H136R&H453R)-CYC1t	This study
pCC83	pRS416-GAL7p-*Bt*CrtI(H136R&N576S)-CYC1t	This study
pCC84	pRS416-GAL7p-*Bt*CrtI(Y160F&H453R)-CYC1t	This study
pCC85	pRS416-GAL7p-*Bt*CrtI(Y160F&N576S)-CYC1t	This study
pCC86	pRS416-GAL7p-*Bt*CrtI(H136R&Y160F&H453R)-CYC1t	This study
pCC87	pRS416-GAL7p-*Bt*CrtI(H136R&Y160F&N576S)-CYC1t	This study
pCC88	pRS416-GAL7p-*Bt*CrtI(H136R&H453R&N576S)-CYC1t	This study
pCC89	pRS416-GAL7p-*Bt*CrtI(Y160F&H453R&N576S)-CYC1t	This study
pCC90	pRS416-GAL7p-*Bt*CrtI(H136R&Y160F&H453R&N576S)-CYC1t	This study

Supplementary Table S6. The codon-optimized sequences of *Tm*CrtE, *Pa*CrtB, and *Bt*CrtI

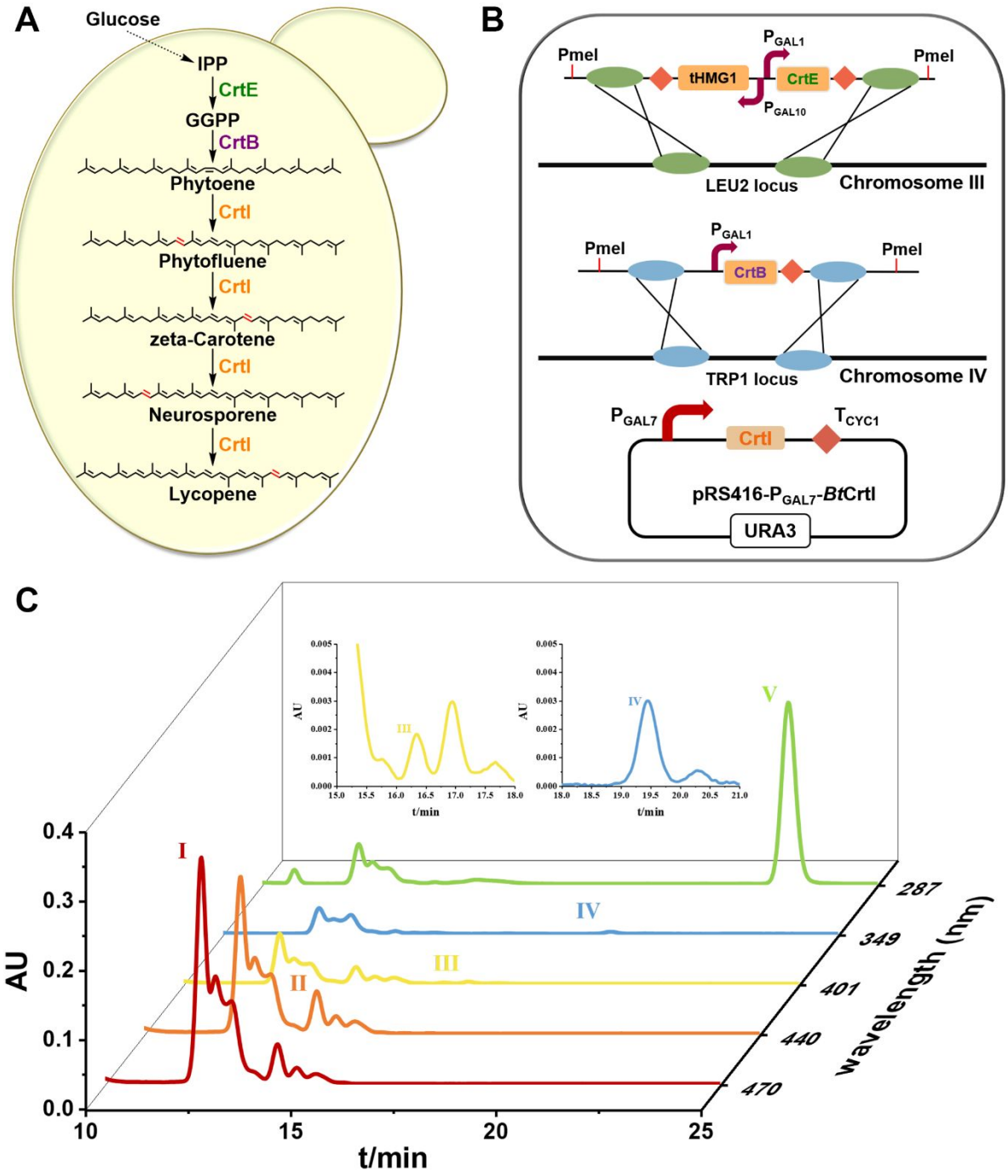
Protein	Encoding sequences
CrtE from *Taxus x media* (*Tm*CrtE)	ATGGCTTATACCGCAATGGCAGCAGGAACTCAGTCATTGCA GTTGAGGACAGTCGCCTCTTACCAGGAGTGCAACTCAATGA GGTCTTGCTTCAAGTTGACCCCATTCAAGTCATTCCACGGTG TCAACTTCAACGTTCCTTCTTTAGGTGCCGCCAACTGCGAAA TCATGGGTCACTTGAAATTGGGTTCTTTGCCATACAAACAGT GTTCAGTATCATCTAAGTCAACTAAGACTATGGCCCAGTTGG TAGATTTGGCAGAGACCGAGAAAGCCGAGGGAAAGGATATC GAGTTCGATTTTAACGAGTATATGAAGTCTAAGGCTGTCGCT GTTGATGCAGCCTTGGATAAGGCCATCCCTTTGGAGTATCCA GAGAAGATCCATGAGTCTATGAGGTACTCATTGTTGGCCGG AGGAAAAAGGGTCAGACCTGCATTATGCATCGCTGCTTGCG AGTTAGTAGGTGGTTCTCAGGACTTGGCCATGCCAACCGCAT GTGCCATGGAAATGATTCATACCATGTCATTGATTCACGATG ATTTGCCTTGCATGGACAACGACGACTTCAGAAGGGGAAAG CCTACCAATCACAAGGTTTTCGGAGAGGACACTGCTGTTTTA GCCGGTGACGCATTGTTATCTTTCGCTTTTGAACACATCGCC GTTGCCACATCAAAAACTGTCCCATCTGACAGGACCTTGAG AGTCATTTCTGAGTTGGGTAAAACCATCGGTTCACAGGGATT GGTCGGAGGTCAGGTAGTCGACATCACTTCTGAGGGAGACG CCAACGTCGACTTAAAGACATTGGAGTGGATTCACATTCAC AAGACTGCCGTCTTGTTGGAATGCTCTGTTGTTTCTGGAGGA ATCTTGGGTGGAGCTACCGAGGATGAGATTGCTAGAATAAG AAGATACGCCAGGTGCGTCGGTTTGTTGTTCCAGGTTGTCGA CGACATTTTGGATGTCACCAAGTCTTCAGAGGAATTGGGAA AGACCGCCGGTAAAGACTTATTGACCGACAAGGCTACCTAC CCTAAGTTGATGGGTTTGGAGAAGGCCAAAGAGTTTGCAGC AGAATTAGCTACCAGGGCAAAGGAAGAGTTGTCATCATTCG ACCAGATCAAGGCAGCCCCTTTGTTAGGATTGGCCGATTACA TCGCTTTCAGGCAAAACTAA
CrtB from *Pantoea agglomerans* (*Pa*CrtB)	ATGTCACAACCACCATTATTGGACCACGCTACACAAACTATG GCAAACGGTTCTAAATCTTTCGCTACTGCTGCTAAATTATTC GACCCAGCAACAAGAAGATCTGTATTGATGTTGTACACCTG GTGTAGACATTGCGATGACGTTATAGATGACCAAACTCACG GTTTTGCTTCAGAAGCTGCAGCCGAAGAAGAAGCTACACAA AGATTGGCAAGATTAAGAACTTTGACATTAGCTGCATTCGA AGGTGCCGAAATGCAAGATCCAGCTTTTGCCGCTTTCCAAGA AGTTGCATTAACCCATGGTATTACTCCTAGAATGGCTTTGGA TCACTTAGACGGTTTTGCAATGGATGTCGCCCAAACAAGATA CGTAACCTTCGAAGACACTTTAAGATATTGTTACCATGTCGC

	CGGTGTTGTCGGTTTGATGATGGCTAGAGTAATGGGTGTTAG AGATGAAAGAGTTTTAGATAGAGCATGTGACTTGGGTTTAG CCTTCCAATTGACAAACATAGCTAGAGATATAATAGATGAC GCAGCCATAGACAGATGCTATTTGCCAGCTGAATGGTTACA AGATGCAGGTTTGACTCCTGAAAATTACGCTGCAAGAGAAA ACAGAGCCGCTTTAGCCAGAGTTGCTGAAAGATTGATAGAT GCAGCCGAACCATATTACATCTCTTCACAAGCTGGTTTGCAT GATTTGCCACCTAGATGCGCATGGGCCATTGCTACCGCAAG ATCTGTTTACAGAGAAATCGGTATTAAAGTCAAGGCTGCAG GTGGTTCCGCATGGGATAGAAGACAACACACTTCTAAAGGT GAAAAGATCGCTATGTTGATGGCCGCTCCTGGTCAAGTTATT AGAGCAAAGACCACCAGAGTCACCCCAAGACCAGCCGGTTT ATGGCAAAGACCTGTTTAA
CrtI from *Blakeslea trispora* (*Bt*CrtI)	ATGTCTGATCAGAAGAAGCACATTGTCGTCATAGGTGCTGG AATAGGAGGTACTGCAACAGCAGCAAGGTTAGCAAGGGAG GGTTTCAGAGTCACTGTCGTCGAGAAGAACGACTTCTCTGGA GGAAGGTGCTCTTTCATTCACCACGACGGTCACAGGTTCGAC CAGGGACCTTCATTGTACTTGATGCCTAAGTTGTTTGAGGAC GCTTTCGCTGACTTAGACGAGAGGATAGGAGACCACTTGGA CTTATTAAGATGTGACAACAATTACAAAGTCCATTTCGACGA CGGTGACGCTGTCCAATTGTCATCAGACTTAACAAAGATGA AGGGTGAGTTGGACAGGATTGAGGGACCTTTAGGATTCGGT AGGTTCTTAGATTTCATGAAAGAGACACACGTCCACTACGA GCAGGGTACATTCATTGCTATAAAGAGAAACTTCGAAACTA TATGGGACTTAATAAGGTTACAGTACGTCCCAGAGATTTTTA GGTTGCACTTATTCGGTAAGATATACGACAGAGCATCAAAA TACTTCCAAACAAAAAAGATGAGGATGGCTTTTACTTTTCAA ACAATGTACATGGGTATGTCACCTTACGACGCACCTGCAGTC TACTCATTGTTGCAATATACAGAGTTCGCAGAGGGAATTTGG TACCCAAGGGGTGGTTTCAACATGGTCGTCCAAAAGTTGGA GTCTATAGCTTCTAAGAAGTACGGAGCTGAGTTCAGGTACC AATCTCCTGTCGCTAAGATTAACACTGTCGATAAAGACAAG AGGGTCACTGGTGTCACTTTGGAGTCTGGAGAAGTCATTGA GGCAGACGCTGTCGTCTGCAACGCTGACTTGGTCTACGCTTA CCACCACTTGTTGCCACCTTGCAACTGGACAAAGAAGACTTT GGCATCTAAGAAATTAACATCTTCATCAATTTCTTTTTACTG GTCAATGTCTACTAAGGTCCCTCAATTGGACGTCCACAACAT TTTCTTGGCTGAGGCTTACAAGGAGTCATTCGACGAGATTTT TAACGATTTCGGTTTGCCTTCTGAAGCATCTTTCTACGTCAA CGTTCCTTCAAGGATAGACGAGTCTGCAGCACCTCCAAATA AGGACTCAATTATAGTTTTAGTTCCAATTGGTCACATGAAGT CTAAGACAGGTAACTCAGCAGAGGAGAACTACCCAGAGTTG GTCAACAGGGCTAGAAAGATGGTCTTGGAGGTCATAGAGAG GAGGTTGGGAGTCAACAACTTCGCTAACTTGATAGAACACG

AGGAGGTCAACGACCCATCAGTCTGGCAATCTAAGTTCAAC
TTGTGGAGGGGATCAATATTAGGTTTATCACATGATGTCTTT
CAGGTTTTGTGGTTCAGACCTTCAACAAAGGACTCTACTAAC
AGATATGACAATTTATTTTTCGTCGGTGCATCAACTCACCCT
GGTACAGGAGTCCCAATAGTCTTGGCAGGATCTAAATTAAC
TTCTGACCAGGTCTGTAAGTCATTCGGACAAAACCCTTTGCC
TAGGAAGTTACAGGACTCTCAGAAGAAATATGCACCTGAGC
AAACAAGGAAGACTGAGTCACACTGGATTTATTACTGCTTA
GCATGCTACTTTGTCACTTTCTTGTTCTTCTATTTCTTTCCTAG
GGACGACACTACTACTCCAGCATCTTTTATTAATCAGTTGTT
GCCAAACGTCTTCCAAGGACAGAACTCTAACGATATTAGGA
TATAA

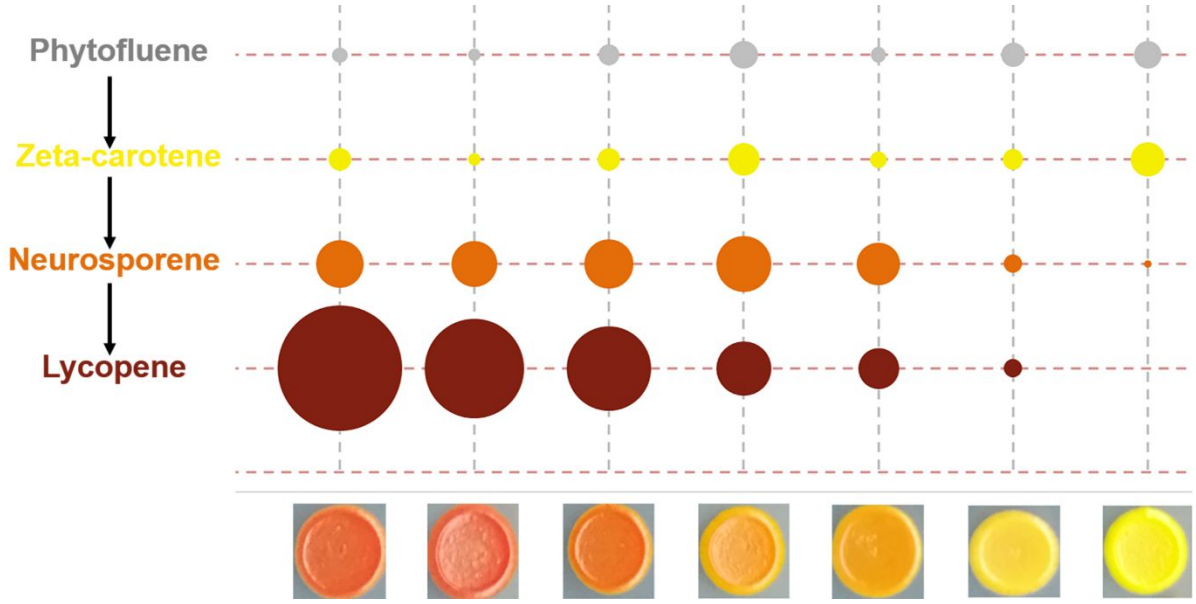
Supplementary Table S7. Codons used for site-specific mutation

amino acid	Codon
C	TGT
G	GGT
S	TCT
K	AAG
Q	CAA
F	TTC
E	GAA
Y	TAC
R	AGG
P	CCA
A	GCT

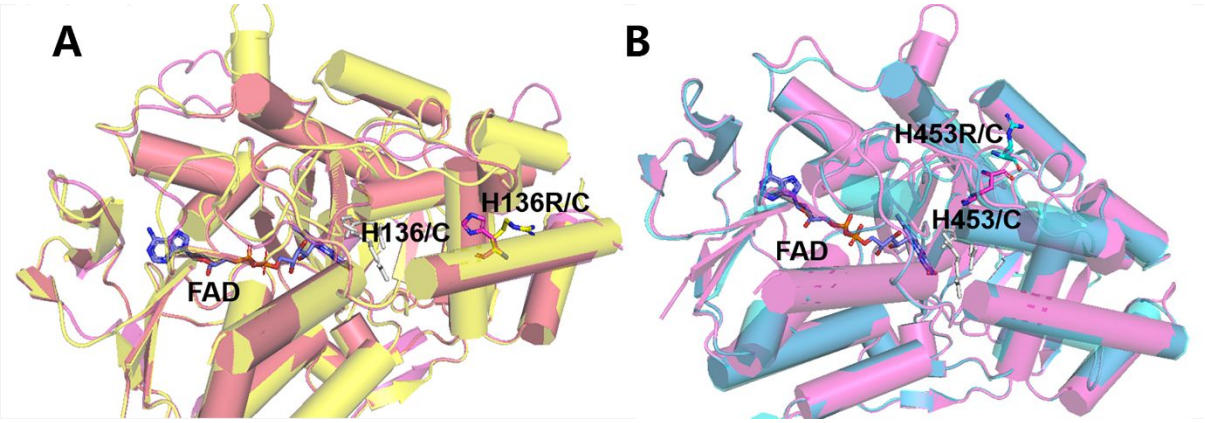


Supplementary Figure S1. Lycopene biosynthetic pathway construction in *S. cerevisiae*. (A) The paradigm of the lycopene biosynthetic pathway in *S. cerevisiae*, which consists of three heterogenous enzymes: CrtE, CrtB, and CrtI. (B) Construction of a lycopene producing strain. The tHMG1, CrtE, and CrtB were integrated into chromosome, while the CrtI was cloned in plasmid and transformed into yeast. (C) The HPLC profile of the wide-type lycopene producing

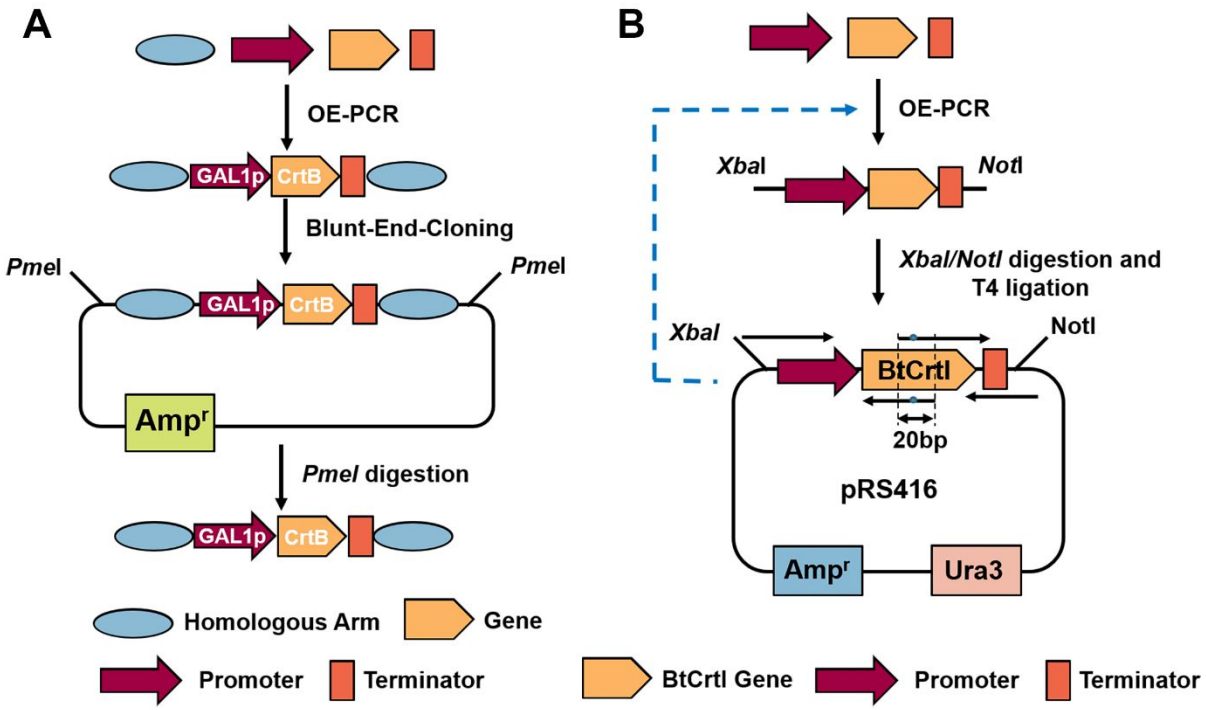
strain (yCC03 in Supplementary Table S1). The signals for lycopene (I), neurosporene (II), ζ-carotene (III), phytofluene (IV), and phytoene (IV) were detected at 470 nm, 440nm, 401 nm, 349 nm, and 287 nm, respectively. CrtE: Geranylgeranyl diphosphate synthase; CrtB: Phytoene synthase; CrtI: Phytoene dehydrogenase; tHMG1: truncated HMG-CoA reductase.



Supplementary Figure S2. The color chart for colony screening. The chart was made by establishing the correspondence between carotenoid accumulation profile and colony color. Circle area indicates the production yield of major carotenoid intermediates, according to the metabolic pathway described on the left.



Supplementary Figure S3. Structural comparison of the wild-type *Bt*CrtI and the two key residue mutants H136R (A) and H453R (B). The models of wild-type *Bt*CrtI, mutants H136R and H453R were colored in purple, yellow, and cyan. The residues H136/H136R and H453/H453R were labeled.



Supplementary Figure S4. Schematic representation of the engineering strategies for CrtB and CrtI expression cassettes. (A) The integrated CrtB expression cassette was assembled by OE-PCR and then cloned into the pEASY-Blunt vector for standby. (B) The CrtI expression cassette was assembled by OE-PCR and then cloned into pRS416 plasmid. The site-specific mutagenesis of *Bt*CrtI was constructed through OE-PCR based on the wide-type *Bt*CrtI expression plasmid.