Front. Chem. Sci. Eng.

DOI 10.1007/s11705-017-1628-0

RESEARCH ARTICLE

Engineering of *β*-carotene hydroxylase and ketolase for astaxanthin overproduction in *Saccharomyces cerevisiae*

Ruizhao Wang[1,2*], Xiaoli Gu[1,2*], Mingdong Yao[1,2], Caihui Pan[1,2], Hong Liu[1,2], Wenhai Xiao(✉)[1,2], Ying Wang(✉)[1,2], Yingjin Yuan[1,2]

1 Key Laboratory of Systems Bioengineering (Ministry of Education), Tianjin University, Tianjin 300072, China

2 SynBio Research Platform, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

Received August 21, 2016; accepted November 12, 2016

E-mails: wenhai.xiao@tju.edu.cn (Xiao W), ying.wang@tju.edu.cn (Wang Y)

* These authors contributed equally to this work

Electronic Supplementary Material

Table S1. Primers used in this study

Primer	Sequences
TDH3p-BDC263Crtw-F	GAATAAACACACATAAACAAACAAAATGTCCGCTGTTACTCCAATG
TDH2t-BDC263Crtw-R	CTAAATCATTAAAGTAACTTAAGGAGTTAAATTTATGAAAATAAAG ACCACCAAGG
TEF1p-AspCrtz-F	ATAGCAATCTAATCTAAGTTTTAATTACAAAATGACACAATTTTTGA TAGTTGTCG
ADH1t-AspCrtz-R	GGCGAAGAAGTCCAAAGCTTTATGAAGGTCTTTCGTCTTGTG
act1-F	CGTTCCAATTTACGCTGGTT
act1-A	GGCCAAATCGATTCTCAAAA
crtW-F	AGCGTGATGAGCGTGATGG
crtW-A	ACTTGGTTGTCCGTAGGTTTATTC
crtZ-F	CTGTCTTGTGGTGGATTGC
crtZ-A	CTTCTAGGTATGTATCTGAATGGC

Table S2. The Codon-optimized sequences of CrtZ and CrtW involved in this study

Protein	Encoding sequences
CrtZ from *Agrobacterium aurantiacum* (AaCrtZ)	atgactaacttcttgatcgttgttgctactgttttggttatggaattgactgcttactctgttcacagatggatc atgcacggtccattgggttggggttggcacaagtctcaccacgaagaacacgaccacgctttggaaaa gaacgacttgtacggtttggttttcgctgttatcgctactgttttgttcactgttggttggatctgggctccag ttttgtggtggatcgctttgggtatgactgtttacggtttgatctacttcgttttgcacgacggtttggttcacc aaagatggccattcagatacatcccaagaaagggttacgctagaagattgtaccaagctcacagattgc accacgctgttgaaggtagagaccactgtgtttctttcggtttcatctacgctccaccagttgacaagttga agcaagacttgaagatgtctggtgttttgagagctgaagctcaagaaagaacttaa
CrtZ from *Alcaligenes sp.*strain PC-1 (AspCrtZ)	atgacacaatttttgatagttgtcgcaaccgtcttggtaatggaattaactgcctattccgttcatagatgga tcatgcacggtccattaggttggggttggcataaaagtcatcacgaagaacatgatcacgccttggaaa agaatgacttgtacggtgtagttttcgctgttttggcaactatcttattcacagttggtgcttactggtggcct gtcttgtggtggattgcattgggtatgaccgtttacggtttgatttacttcatcttgcatgatggtttagtcca ccaaagatggccattcagatacatacctagaagaggttacttcagaagattgtaccaagcccatagatta catcacgctgtagaaggtagagatcattgtgtttcttttggtttcatctacgcaccacctgtcgataaattga agcaagacttaaagagatcaggtgtattaagaccacaagacgaaagaccttcataa
CrtZ from *Brevundimonas sp*. SD212 (BSD212CrtZ)	atggcttggttgacttggattgccttgttcttaacagcatttttgggtatggaagccttcgcttggataatgc atagatatgtcatgcacggtttcttgtggtcctggcatagaagtcatcacgaaccacatgatcacccattg gaaaagaatgacttattcgcagttgtctttgctgcaccagcaatcgtaatggttgccgtcggtttgcatttat ggccttgggccttgcctgttggtttaggtattacagcttatggtatggtatacttttttctttcatgatggtttagt tcacagaagattccctaccggtttttctggtagatcaggtttctggactagaagaatacaagctcatagatt gcatcacgcagtaagaaccagagaaggttgtgtttctttcggtttcttgtgggtcagatcagcaagagcc ttgaaagctgaattagcacaaaagagaggttcttcatccagtggtgcttaa
CrtZ from *Brevundimonas sp*. DC263 (BDC263CrtZ)	atgtcatggccaactatgatcttgttattcttagctacatttttgggtatggaagtctttgcctgggctatgca tagatatgtaatgcacggtttgttatggacttggcatagatcccatcacgaaccacacgatgacgttttag aaagaaatgatttgttcgccgttgtctttgctgcacctgccattatattagtcgctttgggtttacatttgtgg ccttggatgttacctattggtttgggtgtaacagcttatggtttggtttacttttttctttcatgacggtttggttc acagaagattccctaccggtatcgctggtagatcagcattttggactagaagaatacaagcacatagatt acatcacgccgtcagaaccagagaaggttgtgtaagtttcggtttcttgtgggttagatcagcaagagcc ttaaaagctgaattgtctcaaaagagaggttcttcatccaacggtgcataa
CrtZ from *Pantoea agglomerans* (PaCrtZ)	atgttggttaattctttgatagtaatcttgtcagttatagctatggaaggtatcgctgcatttacccatagatat attatgcacggttggggttggagatggcatgaatctcatcacactcctagaaaaggtgtcttcgaattga acgatttatttgccgttgtcttcgctggtgttgctatcgcattaattgcagtcggtactgccggtgtatggcc attgcaatggatcggttgtggtatgacagtttacggtttgttatacttttttagttcatgacggtttggtccacc aaagatggccattccattggatacctagaagaggttatttgaagagattgtacgttgcacacagattacat cacgctgtaagaggtagagaaggttgcgtttcctttggtttcatttacgctagaaagccagctgatttgca agccatattgagagaaagacatggtagaccacctaaaagagacgccgctaaggatagaccagacgc agcctcaccttcttcatccagtccagaataa

CrtZ from *P.stewartii* (PsCrtZ)	atgttgtggatttggaatgccttaatagttttcgtcactgttgtcggtatggaagtagttgctgcattggcac ataaatatataatgcatggttggggttggggttggcatttatcacatcacgaaccaagaaagggtgctttt gaagttaacgacttgtatgctgtcgtattcgcaattgttagtatagctttgatctactttggttctactggtatc tggcctttacaatggattggtgccggtatgacagcttatggtttgttatacttcatggtacatgatggtttagt tcaccaaagatggccattcagatacatacctagaaagggttatttgaagagattgtacatggcacataga atgcatcacgccgtccgtggtaaagaaggttgtgtttcatttggtttcttgtacgctccacctttgtccaagt tacaagcaaccttaagagaaagacacgccgctagatcaggtgcagccagagatgaacaagacggtg ttgatacttcttcatccggtaaataa
CrtZ from *Erwinia uredovora* (EuCrtZ)	atgttgtggatctggaacgctttgatcgtattcgttaccgtcatcggtatggaagttatcgctgcattggca cataaatatattatgcatggttggggttggggttggcatttatctcatcacgaaccaagaaagggtgctttt gaagttaacgatttgtatgcagttgtcttcgccgctttgtctatattgttgatctacttgggttcaactggcat gtggcctttacaatggattggtgccggtatgacagcttatggtttgttatacttcatggtacatgacggtttg gttcaccaaagatggccattcagatacatccctagaaagggttatttgaagagattgtacatggcacata gaatgcatcacgccgtccgtggtaaagaaggttgtgtttccttggtttcttgtacgccccacctttgagta agttacaagctactttaagagaaagacacggtgctagagcaggtgcagccagagatgcacaaggtgg tgaagacgaaccagcctccggtaaataa
CrtZ from *S. solfataricus* P2 (SsP2CtrZ)	atgatgttaatttattacgttggtatggccgttttgacttttgtcggtatggaatttgtcgctagattgatgcat aagtacgtaatgcacggtttgttatggttcatccatgaagatcatcacaaggaaaagcaagcagaattgg aaaagaatgatttgttcggtttagttttcgcctccgttagtgtctatttgtttttcttaggtattcaaggttcttac gtcgctttgtcaatagcaatcggcatgtcttcttatggtatcgcttacttttcatacatgatatggttatccac gacagacatttgcacttaagatcatggggtttgaaacatagaccattcaaggatttgatcttggttcatgac attcatcacaaagaaggcaagggtaactggggtttcttgttcgtaatcaagggtttagataaggttcctat cttgaaagacgaataa
Chyb from *Haematococcus pluvialis* (HpChyb)	atgttgtccaagttgcaatccatctccgttaaggctagaagagttgaattggctagagacatcaccagac caaaagtttgcttacacgctcagagatgctctttggtcagattgagagttgcagctccacaaacagaaga agcagttggaactcaacaagcagcaggagcaggagacgaacattcagcagacgttgctttgcaacaa ttggatagagctatcgcagaaagaagagctagaaggaagagagaacaattgtcttaccaagccgctg ctattgcagcttctattggagtttcaggtattgctatcttcgctacctacttgagatttgctatgcatatgaca gttggaggagcagttccttggggagaagttgcaggtccattattattggtggttggaggtcaattgggtat ggaaatgtacgctagatacgctcataaggctatttggcacgaatctccattaggttggctattgcacaagt ctcatcatactccaagaaccggtccatttgaagctaacgatttgttcgctatcatcaagggtctaccagct atgttgttgtgtactttcggcttttggttgccaaacgttttaggtacagcttgtttcggagcaggattaggcat tactttatacggcatggcttacatgttcgttcacgacggtttagtttccagaagatttccaacaggtccaatt gcaggtttaccatacatgaagaggttgacagttgctcaccaattgcatcattcaggaaaatacggtgga gcaccttggggaatgtttttaggtccacaagaattgcaacatattccaggagcagcagaagaagttgaa agattggtgttggagttggattggtctaagaggtaa

CrtW from *Gloeobacter violaceus* PCC 7421 (GvCrtW)	atgatgagaggttctgctgttaaagaaagaacatcaaagagattagcagaaggtgtcattacccataaa aatgattcttcaggtttgtggtgggcattggttataatcggtttgtggatattttccttcgctgctgctttgaga ttaccaatcggtgaattgagtttacaagccgttatcggtgttgtcattttgagaacatttttgcatacaggttt gttcattaccgcccatgatgctatgcacagaactgtatttccagccaatcacagaataaacgactggtta ggtactgctgctgttggtttgtatgctttcatgccttacagagaattgttgatcaagcatcaattacatcaca gattcccagcaaccggtaaagatccagactatcatgatggtgaacactctggtttctttcaatggtatttaa agttcatgaaggactacatggaatcaagaaacactccatttttgatcgctggtatggccgtagttttcggt gtttgtacatggttaatgggtgtcccattggtaaacttggcattattctggttgttacctttggtcttatccagt ttgcaattgttttatttcggtacttacttaccacatagacaacctgatggtggttatagaaatagacacagag ctacttccaacagattgtcttcattttggtccttcgtcagttgctatcattttggttaccactgggaacatcac gaatacccattagtaccttggcatagattgcctgaagctagaagataa
CrtW from *Agrobacterium aurantiacum* (AaCrtW)	atgtccgcacatgccttgccaaaagcagacttaactgccacatccttgattgtcagtggtggtattatagc tgcatggttagctttgcatgtacacgcattgtggtttttagatgccgctgcacatcctatattagctatcgca aatttcttgggtttgacatggttgtctgttggtttattcatcattgcccatgacgctatgcacggttcagttgtc ccaggtagacctagagcaaacgccgctatgggtcaattggttttgtggttgtacgctggtttctcttggag aaaaatgatagtcaagcacatggcccatcacagacacgctggtactgatgacgatccagacttcgatc atggtggtcctgtcagatggtatgctagatttattggtacatacttcggttggagagaaggtttgttattgcc agtcatcgtaaccgtttatgcattgattttaggtgatagatggatgtacgtagttttctggccattgccttcta tcttggcttcaatccaaatgttcgttttcggtacttggttgccacatagacctggtcacgacgcattcccag atagacataatgccagatcatcaagaatttccgatcctgtaagtttattgacttgtttccatttcggtggttac catcacgaacatcacttacacccaactgttccttggtggagattgccatctaccagaactaagggtgata cagcttaa
CrtW from *Alcaligenes sp.*strain PC-1 (AspCrtW)	Atgtctggtagaaaaccaggtactacaggtgacactattgtcaatttaggtttgacagctgcaatattgtt atgttggttggttttgcatgcatttactttgtggttgttagatgccgctgcacatcctttgttagccgtattatg cttggctggtttgacatggttgtctgttggtttatttattatcgcacatgatgccatgcacggttcagttgtcc caggtagacctagagctaatgccgctatcggtcaattagcattgtggttatatgccggtttttcctggcca aaattgattgctaagcacatgacccatcacagacacgcaggtactgataacgacccagatttcggtcat ggtggtcctgttagatggtatggttcttttgtctcaacctacttcggttggagagaaggtttgttattgcctgt aatagttaccacttatgcattgatcttaggtgatagatggatgtacgttatattttggccagtccctgctgtat tggcatcaatccaaatttttgttttcggtacatggttaccacatagacctggtcacgatgacttcccagaca gacataacgccagatccacaggtattggtgatcctttaagtttattgacctgtttccatttcggtggttacca tcacgaacatcacttgcatccacacgttccttggtggagattaccaagaacaagaaagaccggtggtag agcttaa
CrtW from *Brevundimonas sp*. DC263 (BDC263CrtW)	atgtccgctgttactccaatgagtagagttgtccctaatcaagcattgattggtttgactttagcaggtttga ttgctgctgcttggttgacattgcatatatacggtgtctacttccacagatggacaatctggtctgttttgac cgtcccattaattgtagctggtcaaacttggttgtccgtaggtttattcatagttgctcatgatgcaatgcac ggtagtttggcaccagccagacctagattaaacacagcaatcggttctttggctttagcattgtatgccgg ttttagattcaccccattgaaaactgcccatcacgctcatcacgctgcaccaggtacagcagatgaccct gattttcatgccgacgctccaagagcatttttgccttggttctatggtttctttagaacctacttcggttggag agaattagcagttttgactgtattagttgcagtcgccgtattaatattgggtgctagaatgccaaatttgtta gtcttttgggccgctcctgcattgttgtctgccttacaattgttcactttcggtacatggttgccacatagac

acaccgatgacgcctttcctgataatcataacgctagaacatctccattcggtcctgttttatcattgttgac
ctgtttccatttcggtagacatcacgaacatcacttgacaccttggaagccttggtggtctttattttcataa

CrtW from *Brevundimonas sp.* SD212 (BSD212CrtW)

atgactgctgctgttgctgaaccaagaatcgttcctagacaaacctggattggtttgactttagctggtatg
atagttgcaggttggggttctttacatgtctatggtgtatactttcatagatggggtacatcttcattggttatt
gtcccagctatagtagcagttcaaacctggttatctgttggtttgttcattgtcgcccatgatgctatgcacg
gttcattagctccaggtagacctagattgaatgccgctgttggtagattgacattaggtttgtatgcaggttt
cagattcgatagattgaagacagcacatcacgcccatcacgcagccccaggtactgctgatgaccctg
acttttatgccccagctcctagagcatttttaccttggttcttgaatttctttagaacttacttcggttggagag
aaatggccgtattgacagctttggttttgatcgctttgttcggtttgggtgctagaccagcaaacttgttaac
tttctgggctgcacctgcattgttgtcagccttacaattgttcactttcggtacatggttaccacatagacac
acagatcaacctttgcagacgcccatcacgctagatccagtggttacggtcctgttttgtccttgttaacc
tgttttcatttcggtagacatcacgaacatcacttaactccttggaggccttggtggagattgtggagagg
tgaaagttaa

BKT from *Chlamydomonas reinhardtii* (CrBKT)

atgggtccaggaattcaaccaacttcagctagaccatgttctagaaccaagcattccagattcgctttatt
ggcagcagctttaacagctagaagggttaagcagttcaccaagcaattcagatccagaagaatggcag
aagacatcttgaagctttggcaaagacaataccacttgccaagagaagattccgataagagaaccttga
gggaaagagttcacttgtacagaccaccaagatcagatttgggtggtattgcagttgcagttacagttatt
gccctttgggctactttgttcgtttacggtctttggttcgttaagttgccttgggctttgaaagttggagaaac
agctacttcttgggctactattgcagcagttttcttctctttggagttcttgtacaccggattgttcattactact
cacgacgctatgcacggtactattgccttgagaaacaggagattgaacgacttcttgggtcaattggcta
tctccttgtacgcttggttcgattattccgttttgcataggaagcattgggaacatcataatcataccggcg
aaccaagagttgatccagatttccataggggtaatcctaatttggcagtttggttcgctcaattcatggtttc
ctacatgaccttgtcccaatttttgaagatcgccgtttggtccaacttattgttgttagcaggagctccattg
gctaatcaattgttgttcatgaccgcagctccaattttgtccgccttcagattgttctactacggtacttacgt
cccacatcatccagaaaaaggtcatacaggagctatgccttggcaagtttcaagaacttcttccgcttcc
agattgcaatctttcttgacttgctaccacttcgatttgcattgggaacaccataggtggccatacgctcctt
ggtgggaattacctaagtgtagacagattgcaagaggagcagctttagctccaggtccattaccagttc
cagcagcagcagcagctacagcagctacagcagcagcagcagcagcagctacaggttctccagctc
cagcttctagagcaggttcagcttcttcagcttcagcagcagcttcaggttttggttcaggtcattcaggtt
cagttgcagctcaaccattgtcttctttgccattgttgtcagaaggagttaagggtttggttgaaggagcta
tggaattagttgcaggaggttcttcttcaggaggtggtggtgaaggaggtaaaccaggagcaggagaa
cacggtttattgcaaagacaaaggcaattggctccagttggagttatggcttaa

CrtW from *Nostoc punctiforme* PCC 73102 (NpCrtW)

atgattcaattggaacaaccattatcccatcaagccaaattgacacctgtcttgagatcaaagtcacaatt
caagggtttgttcatagctatcgtaattgtttcagcatgggtaatttccttaagtttgttgttgtctttggatatc
tcaaaattgaagttttggatgttattgccagttatcttgtggcaaaccttttgtacactggtttattcattactt
ctcacgacgcaatgcatggtgttgtcttcccacaaaacacaaagatcaaccacttgatcggtactttgac
attgtcattatacggtttattgccttaccaaaagttattgaaaaagcattggttgcatcaccataatccagca
tcttcaattgatcctgactttcacaacggtaaacatcaatccttttcgcctggtattttcatttcatgaagggt
tactggagttggggtcaaataatcgccttgaccatcatctacaacttcgctaagtacatcttgcatatccca
tctgataacttgacttacttctgggttttaccttcattattgtccagtttgcaattgttttatttcggtacatttttg
ccacactctgaacctataggtggttacgtccaaccacattgtgctcaaacaatctccagacctatttggtg
gagttttattacctgctatcacttcggttaccatgaagaacaccatgaatatccacatatatcttggtggca

	attacctgaaatctacaaagcaaagtaa
CrtW from *Sphingomonas* sp. DC18 (SDC18CrtW)	atgactgttgatcatgacgctagaatatctttgttattggctgctgctatcggtgctgcatggttagccatcc atgtcggtgctattgtatggtggagatggtcaccagctactgctgttttagctattcctgttgtcttggtaca agcatggttatccacaggtttgtttattgttgctcatgattgtatgcacggtagtttcgtcccaggtagacca gctgttaatagaactgtcggtacattatgcttgggtgcctatgctggtttatcctacggtcaattgcatccaa aacatcacgctcatcacgacgcaccaggtacagccgctgatcctgactttcatgcaggtgccccaaga tcagcattaccttggttcgccagatttttcacctcatactacactcatggtcaaattttaagaataaccgcag ccgctgtattgtacatgttgttgggtgtttctttattgaacatcgtagttttctgggcattaccagccttgatag ctttagcacaattgtttgttttcggtacatttttgccacatagacacggtgataccccttcgccgacgctca taatgctagatccaacggttggcctagattagcaagtttggttacctgttttcacttcggtgcttaccatcac gaacatcacttatctccttggactccttggtggcaattgccaagagttggtcaaccagctgctggtagaa gatcattgtctaaggatagataa

Table S3. Plasmids used in this study

Plasmids	Description	Source
pLD2	Blunt Cloning vector, resistant to ampicillin	This Lab
pUC57-Simple	Blunt Cloning vector, resistant to ampicillin	GenScript
pRS425K	Multiple copy plasmid in *S.cerevisiae* with *LEU2* and *KanMX* marker	This Lab
pUC57-Simple-01	Gene *crtZ* from *Agrobacterium aurantiacum* (*AacrtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-02	Gene *crtZ* from *Alcaligenes sp.* strain PC-1 (*AspcrtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-03	Gene *crtZ* from *Brevundimonas sp.* SD212 (*BSD212crtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-04	Gene *crtZ* from *Brevundimonas sp.* DC263 (*BDC263crtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-05	Gene *crtZ* from *Pantoea agglomerans* (*PacrtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-06	Gene *crtZ* from *Erwinia uredovora* (*EucrtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-07	Gene *crtZ* from *Sulfolobus solfataricus* P2 (*SsP2crtZ*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-08	Gene *chyb* from *Haematococcus pluvialis* (*Hpchyb*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-09	Gene *crtZ* from *Pantoea stewartii (PscrtZ)* was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-10	Gene *crtW* from *Gloeobacter violaceus* PCC 7421 (*GvcrtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-11	Gene *crtW* from *Agrobacterium aurantiacum* (*AacrtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-12	Gene *crtW* from *Alcaligenes sp.* strain PC-1 (*AspcrtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study

pUC57-Simple-13	Gene *crtW* from *Brevundimonas sp.* SD212 (*BSD212crtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-14	Gene *crtW* from *Brevundimonas sp.* DC263 (*BDC263crtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-15	Gene *bkt* from *Chlamydomonas reinhardtii* (*Crbkt*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-16	Gene *crtW* from *Nostoc punctiforme PCC 73102* (*NpcrtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pUC57-Simple-17	Gene *crtW* from *Sphingomonas* sp. DC18 (*SDC18crtW*) was codon optimized, synthesized and cloned into pUC57-Simple	This study
pLD2-01	The cassette ADH1t-FBA1p-TDH3p-TDH2t was synthesized and inserted into the PstI/BamHI site of pLD2	This study
pLD2-02	Gene *GvcrtW* was digested from pUC57-Simple-10 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-03	Gene *AacrtW* was digested from pUC57-Simple-11 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-04	Gene *AspcrtW* was digested from pUC57-Simple-12 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-05	Gene *BSD212crtW* was digested from pUC57-Simple-13 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-06	Gene *BDC263crtW* was digested from pUC57-Simple-14 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-07	Gene *Crbkt* was digested from pUC57-Simple-15 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-08	Gene *NpcrtW* was digested from pUC57-Simple-16 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-09	Gene *SDC18crtW* was digested from pUC57-Simple-17 by BsaI and inserted into the same site of pLD2-01	This study
pLD2-10	Gene *AacrtZ* was cut from pUC57-Simple-01 by BsaI and inserted into BsmBI site of pLD2-03	This study
pLD2-11	Gene *AspcrtZ* was cut from pUC57-Simple-02 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-12	Gene *BDC263crtZ* was cut from pUC57-Simple-04 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-13	Gene *BSD212crtZ* was cut from pUC57-Simple-03 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-14	Gene *EucrtZ* was cut from pUC57-Simple-06 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-15	Gene *PacrtZ* was cut from pUC57-Simple-05 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-16	Gene *SsP2crtZ* was cut from pUC57-Simple-07 by BsaI and inserted into BsmBI site of pLD2-11	This study
pLD2-17	Gene *Hpchyb* was cut from pUC57-Simple-08 by BsaI and inserted into BsmBI site of pLD2-04	This study

pLD2-18	Gene *AspcrtZ* was cut from pUC57-Simple-02 by BsaI and inserted into BsmBI site of pLD2-04	This study
pLD2-19	Gene *Hpchyb* was cut from pUC57-Simple-08 by BsaI and inserted into BsmBI site of pLD2-05	This study
pLD2-20	Gene *AspcrtZ* was cut from pUC57-Simple-02 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-21	Gene *SsP2crtZ* was cut from pUC57-Simple-07 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-22	Gene *PscrtZ* was cut from pUC57-Simple-09 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-23	Gene *Hpchyb* was cut from pUC57-Simple-08 by BsaI and inserted into BsmBI site of pLD2-02	This study
pLD2-24	Gene *EucrtZ* was cut from pUC57-Simple-06 by BsaI and inserted into BsmBI site of pLD2-02	This study
pLD2-25	Gene *PacrtZ* was cut from pUC57-Simple-05 by BsaI and inserted into BsmBI site of pLD2-02	This study
pLD2-26	Gene *BDC263crtZ* was cut from pUC57-Simple-04 by BsaI and inserted into BsmBI site of pLD2-02	This study
pLD2-27	Gene *Hpchyb* was cut from pUC57-Simple-08 by BsaI and inserted into BsmBI site of pLD2-07	This study
pLD2-28	Gene *PacrtZ* was cut from pUC57-Simple-05 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-29	Gene *EucrtZ* was cut from pUC57-Simple-06 by BsaI and inserted into BsmBI site of pLD2-07	This study
pLD2-30	Gene *PscrtZ* was cut from pUC57-Simple-09 by BsaI and inserted into BsmBI site of pLD2-03	This study
pLD2-31	Gene *Ssp2crtZ* was cut from pUC57-Simple-07 by BsaI and inserted into BsmBI site of pLD2-05	This study
pLD2-32	Gene *BSD212crtZ* was cut from pUC57-Simple-03 by BsaI and inserted into BsmBI site of pLD2-05	This study
pLD2-33	Gene *EucrtZ* was cut from pUC57-Simple-06 by BsaI and inserted into BsmBI site of pLD2-05	This study
pLD2-34	Gene *HpChyb* was cut from pUC57-Simple-08 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-35	Gene *EucrtZ* was cut from pUC57-Simple-06 by BsaI and inserted into BsmBI site of pLD2-06	This study
pLD2-36	Gene *BSD212crtZ* was cut from pUC57-Simple-03 by BsaI and inserted into BsmBI site of pLD2-02	This study
pLD2-37	Gene *BDC263crtZ* was cut from pUC57-Simple-04 by BsaI and inserted into BsmBI site of pLD2-09	This study
pLD2-38	Gene *AspcrtZ* was cut from pUC57-Simple-02 by BsaI and inserted into BsmBI site of pLD2-08	This study
pLD2-39	Gene *PscrtZ* was cut from pUC57-Simple-09 by BsaI and inserted into BsmBI site of pLD2-05	This study

pLD2-40	The cassette ADH1t-TEF1p-TDH3p-TDH2t was synthesized and inserted into PstI/BamHI site of pRS425K	This study
pLD2-41	Gene *BDC263crtW* was digested from pUC57-Simple-14 by BsaI and inserted into the same site of pLD2-40	This study
pLD2-42	Gene *AspcrtZ* was cut from pUC57-Simple-02 by BsaI and inserted into BsmBI site of pLD2-41	This study
pWRZ01	The cassette ADH1t-*AacrtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-10 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ02	The cassette ADH1t-*AspcrtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-11 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ03	The cassette ADH1t-*BDC263crtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-12 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ04	The cassette ADH1t-*BSD212crtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-13 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ05	The cassette ADH1t-*EucrtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-14 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ06	The cassette ADH1t-*PacrtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-15 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ07	The cassette ADH1t-*SsP2crtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-16 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ08	The cassette ADH1t-*Hpchyb*-FBA1p-TDH3p-*AspcrtW*-TDH2t were digested from pLD2-17 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ09	The cassette ADH1t-*AspcrtZ*-FBA1p-TDH3p-*AspcrtW*-TDH2t were digested from pLD2-18 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ10	The cassette ADH1t-*Hpchyb*-FBA1p-TDH3p-*BSD212crtW*-TDH2t were digested from pLD2-19 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ11	The cassette ADH1t-*AspcrtZ*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-20 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ12	The cassette ADH1t-*SsP2crtZ*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-21 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ13	The cassette ADH1t-*PscrtZ*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-22 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ14	The cassette ADH1t-*Hpchyb*-FBA1p-TDH3p-*GvcrtW*-TDH2t were digested from pLD2-23 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ15	The cassette ADH1t-*EucrtZ*-FBA1p-TDH3p-*GvcrtW*-TDH2t were digested	This study

	from pLD2-24 by PstI/BamHI and inserted into the same site of pLD2-01	
pWRZ16	The cassette ADH1t-*PacrtZ*-FBA1p-TDH3p-*GvcrtW*-TDH2t were digested from pLD2-25 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ17	The cassette ADH1t-*BDC263crtZ*-FBA1p-TDH3p-*GvcrtW*-TDH2t were digested from pLD2-26 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ18	The cassette ADH1t-*Hpchyb*-FBA1p-TDH3p-*CrBKT*-TDH2t were digested from pLD2-27 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ19	The cassette ADH1t-*PacrtZ*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-28 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ20	The cassette ADH1t-*EucrtZ*-FBA1p-TDH3p-*CrBKT*-TDH2t were digested from pLD2-29 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ21	The cassette ADH1t-*PscrtZ*-FBA1p-TDH3p-*AacrtW*-TDH2t were digested from pLD2-30 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ22	The cassette ADH1t-*Ssp2crtZ*-FBA1p-TDH3p-*BSD212crtW*-TDH2t were digested from pLD2-31 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ23	The cassette ADH1t-*BSD212crtZ*-FBA1p-TDH3p-*BSD212crtW*-TDH2t were digested by pLD2-32 with PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ24	The cassette ADH1t-*EucrtZ*-FBA1p-TDH3p-*BSD212crtW*-TDH2t were digested from pLD2-33 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ25	The cassette ADH1t-*HpChyb*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-34 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ26	The cassette ADH1t-*EucrtZ*-FBA1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-35 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ27	The cassette ADH1t-*BSD212crtZ*-FBA1p-TDH3p-*GvcrtW*-TDH2t were digested from pLD2-36 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ28	The cassette ADH1t-*BDC263crtZ*-FBA1p-TDH3p-*SDC18crtW*-TDH2t were digested from pLD2-37 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ29	The cassette ADH1t-*AspcrtZ*-FBA1p-TDH3p-*NpcrtW*-TDH2t were digested from pLD2-38 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ30	The cassette ADH1t-*PscrtZ*-FBA1p-TDH3p-*BSD212crtW*-TDH2t were digested from pLD2-39 by PstI/BamHI and inserted into the same site of pLD2-01	This study
pWRZ31	The cassette ADH1t-*AspcrtZ*-TEF1p-TDH3p-*BDC263crtW*-TDH2t were digested from pLD2-42 by PstI/BamHI and inserted into the same site of pLD2-01	This study

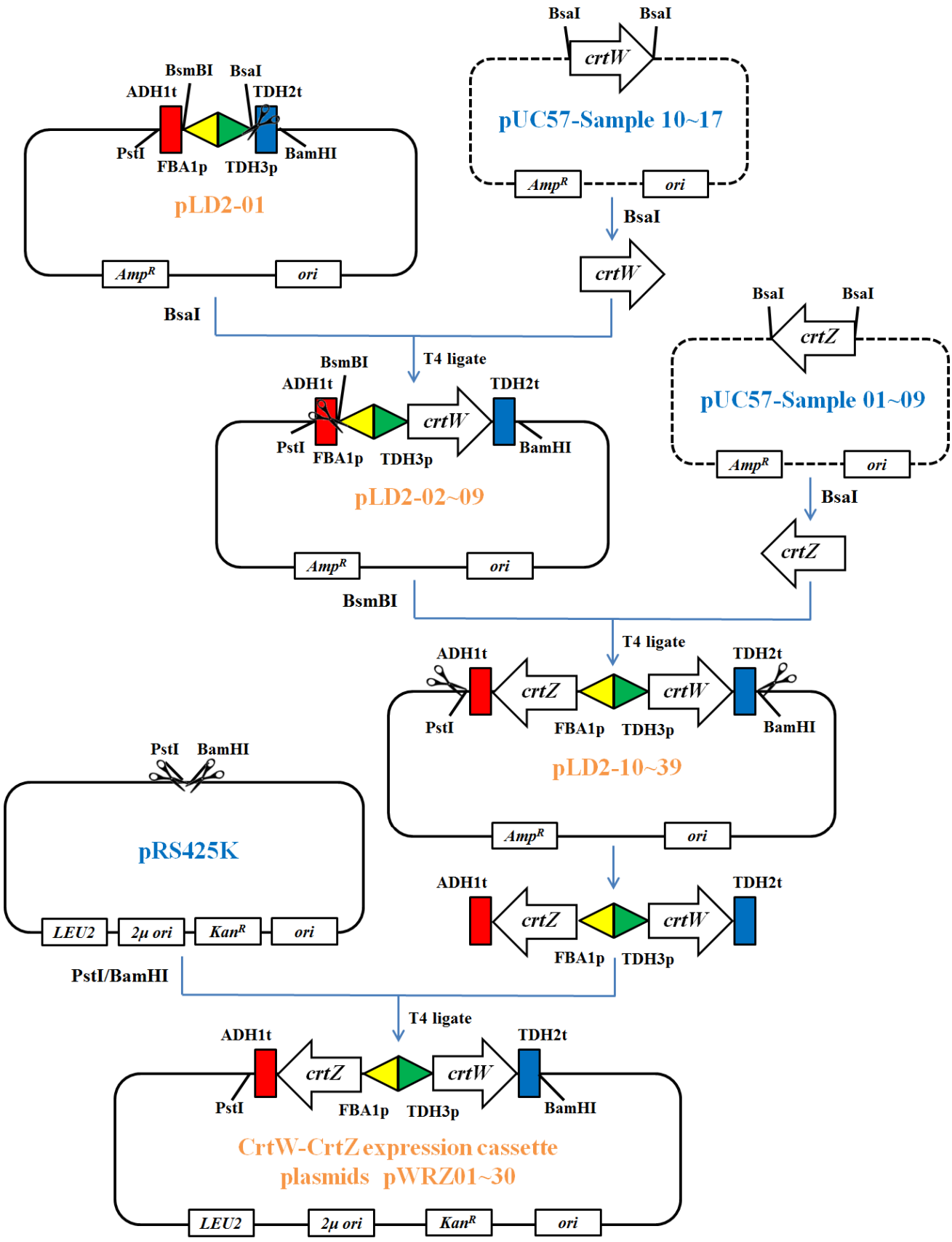

Fig. S1 Construction of Crtw-CrtZ expression cassette plasmids.

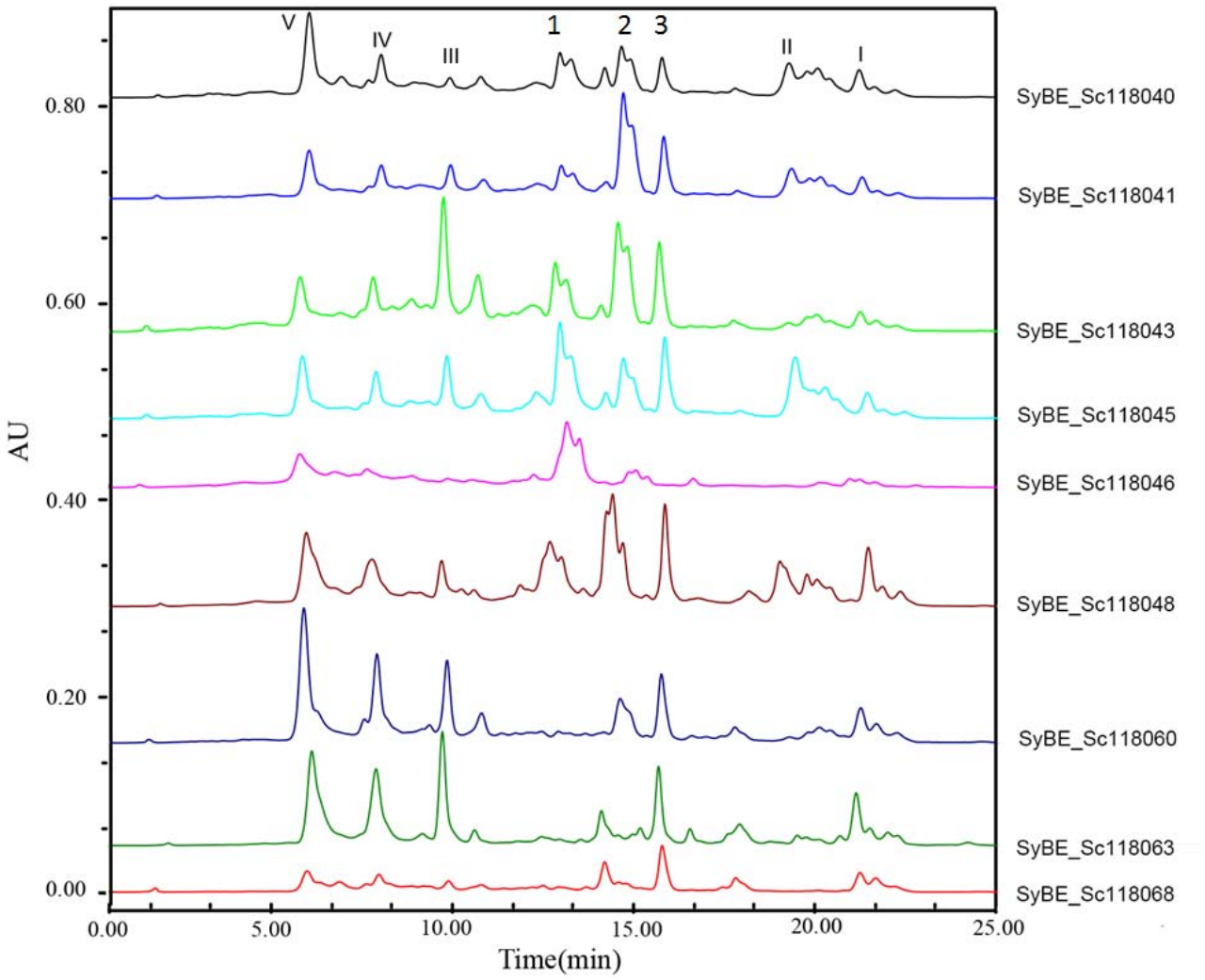

Fig. S2 HPLC analysis of astaxanthin producing strains. I, β-carotene; II, lycopene; III, canxanthathin; IV, zeaxanthin; V, astaxanthin.

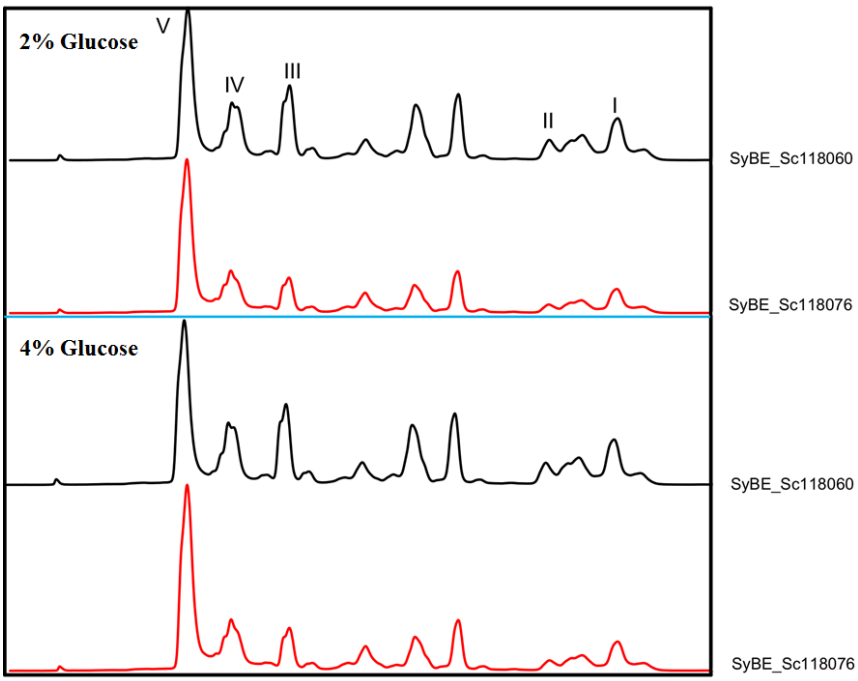

Fig. S3 HPLC analysis of engineered strains with/without alternation on promoter activity of *crtZ*. I, β-carotene; II, lycopene; III, canxanthathin; IV, zeaxanthin; V, astaxanthin