



Figure S1. The phenotypic observation of p1300BZ, p1300BZ+p1300BR, with Mock as a control.

Table S1. Amino acid and DNA sequences of studied genes and promoters.

Name	Sequence
<i>CrtW</i> gene (Amino acid sequence)	MTAAVAEPRIVPRQTWIGLTLAGMIVAGWGS�HVYGVYFHRWGTSS LVIVPAIVAVQTWLSVGLFIVAHDA MHGSLAPGRPRLNAAVGRLTL GLYAGFRFDRLKTAHHAHHAAPGTADDPDFYAPAPRAFLPWFLNF FRTYFGWREMAVLTA LVIALFGLGARPANLLTFWAAPALLSALQLF TFGTWLP HRHTDQPFADAH HARSSGYGPVLSLLTCFHFG RHHEHHL TPWRPWWRLWRGES
<i>CrtW</i> gene (Optimized sequence)	ATGGCTTGGTTAACATGGATTGCATTGTTTCTTACTGCATTTCTTG GAATGGAAGCATTGCTTGGATTATGCATAGATATGTGATGCAT GGATTTCTTTGGAGTTGGCATAGGTCACATCATGAACCACATGAT CATCCTTTAGAAAAAATGATTTATTTGCTGTTATTTTGGCTGCTC CAGCAATTGTTATGGTGGCAGTGGGACTTCATCTTTGGCCTTGGG CACTTCCAGTGGGTTTAGGTATTACAGCTTATGGTATGGTGTATT TTTTTTTTCATGATGGACTTGTGCATAGGAGGTTTCCTACAGGATT TAGTGGTAGATCAGGATTTTGGACAAGGCGTATACAAGCACATA GGCTTCATCATGCAGTGAGAACTAGAGAAGGTTGTGTGTCATTT GGTTTCTTTGGGTGAGGTCTGCTAGAGCACTTGAAGCTGAATTA GCTCAAAAAAGGGGAAGTAGTAGTTCAGGAGCTTAATGA
<i>CrtZ</i> gene (Amino acid sequence)	MAWLTWIALFLTAFLGMEAFAWIMHRYVMHGFLWSWHRSHHEPH DHPLEKNDLFAVIFAAPAIVMVA VGLHLWPWALPVGLGITAYGMVYF FFHDGLVHRRFPTGFSGRSGFWTRRIQAHRLHHAVRTREGCVSFGFL WVRSARALEAELAQKRGSSSSGA
<i>CrtZ</i> gene (Optimized sequence)	ATGGCTTGGTTAACATGGATTGCATTGTTTCTTACTGCATTTCTTG GAATGGAAGCATTGCTTGGATTATGCATAGATATGTGATGCAT GGATTTCTTTGGAGTTGGCATAGGTCACATCATGAACCACATGAT CATCCTTTAGAAAAAATGATTTATTTGCTGTTATTTTGGCTGCTC

	CAGCAATTGTTATGGTGGCAGTGGGACTTCATCTTTGGCCTTGGG CACTTCCAGTGGGTTTAGGTATTACAGCTTATGGTATGGTGTATT TTTTTTTTCATGATGGACTTGTGCATAGGAGGTTTCCTACAGGATT TAGTGGTAGATCAGGATTTTGGACAAGGCGTATACAAGCACATA GGCTTCATCATGCAGTGAGAACTAGAGAAGGTTGTGTGTCATTT GGTTTTCTTTGGGTGAGGTCTGCTAGAGCACTTGAAGCTGAATTA GCTCAAAAAAGGGGAAGTAGTAGTTCAGGAGCTTAATGA
35 S promoter sequence	TGAGACTTTTCAACAAAGGGTAATATCCGGAACCTCCTCGGAT TCCATTGCCCAGCTATCTGTCACTTTATTGTGAAGATAGTGAAAA AGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATG GACCCCCACCCACGAGGAGCATCGTGAAAAAGAAGACGTTCC AACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTG ACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCT TCCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGGAC
rbcs promoter sequence	TGTGGGAACGAGATAAGGGCGAAGTGCGCTAGTAGCCTGCTATT TAAAATATATCCACAATTTATAATGTATTTGAAGATTAGTCAATT CGTCCAAAATTCAGGACTAAGTATCTTGAATTTTTGTATCCTGAA TTTTGGGGCTACTAATTTGGAACCTCAGGACTTAATGTCCTAAATT TTTGAGCCGCTAATTTGAAATTCAGGACTAAGTGTTTTGAATTTTT GAACTGCTTATTCGAAATGCAAGACTAAGTGACATGGATTTTTG AACTGCTAATTTAATATTCAGGACATAAGATTCTGAATTTTCAGAC ATAATTTTTTAACCTTAGGGCACGATGTCCTGAAGTTTGAATCTT GAGGTCTAAACTTCAAGATGCAGCGTCTTGAAGTTTAAAGTGAAC TGGCTAATCTTTAAATACTTGTAAACTGTGGATACATTTTTAAAT AATATATTTAAAAGCGGTACCTGGTATCATCTTCACGAGAATTT TCCAAGTTAATTGTAAAGGAAATAGTGGTGTTGCATCAAGTTAT GGACAATATAAGGAAGCAAACAGTACTCTAGCTATCAAATTAGT TTCCACTTCTAAACCATGAATATTAGGAAAAACAAGAAACAAA ACAAATATACATAAACAATACGGCTAAAGCCCAAGGAAAAGGG ACTCTAAAAAAATTAACCAACCTCAATCACACATTCATATCCTC TTCTACCTCATCTAGGATGAGATAAGATTACTGAGGTGCTTACA CGTGGCACCTCCATTGTGGTGACTAAATGAAGAGTGGCTTAGCT CAAAATATAATTTCCAACCTTTCATGTGTGGATATTAAGTTTTGT GTAGTGAATCAAGAACCACATAATCCAATGGTTAGCTTTATTCC AAGATGAGGGGGTTGTTGATTTTTGTCCGTCAGATATAGGAAAT ATGTAAAACCTTATCATTATATATAGGGTGGTGGGCAACTATGC AATGACCATATTGGAAGTTAAAGGAAAAGAGAGAAAGAGAAAT CTTCTGAATCGTCGACCTGCAGGCATG
TP sequence	TGGCTTCCTCTGTCATTTCTTCAGCAGCTGTTGCCACACGCAGCA ATGTTACACAAGCTAGCATGGTTGCACCTTTCAGTGGTCTCAAAT CTTCAGCCACTTTCCCTGTTACAAAGAAGCAAAACCTTGACATC ACTTCCATTGCTAGCAATGGTGGAAGAGTTAGCTGC
p1300BGFP	CGATCGGTGCGATTCATAGAAGATTAGATTTTTTCATAGTATTTTTTT

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TTCATATATATTTGCTTTAAAAAAAATTGACAATCCATTTCGTTT
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TTCCTTATATCATGCATGGTTTCACATATATCAAAGGATAAAAGC
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 CATCGTTACAATCAACATGCTACCCTCCGCGAGATCATCCGTGTT
 TCAAACCCGGCAGCTTAGTTGCCGTTCTTCCGAATAGCATCGGTA
 ACATGAGCAAAGTCTGCCGCTTACAACGGCTCTCCCGCTGACG
 CCGTCCCGGACTGATGGGCTGCCTGTATCGAGTGGTGATTTGTG
 CCGAGCTGCCGGTCGGGGAGCTGTTGGCTGGCTGGTGGCAGGAT
 ATATTGTGGTGTAACAATTGACGCTTAGACAACCTTAATAACA
 CATTGCGGACGT

Table S2. Codon frequency table of *Lactuca sativa*.

Codon	RSCU	Codon	RSCU	Codon	RSCU	Codon	RSCU
TTT	0.529	TTC	0.471	TTA	0.163	TTG	0.244
TCT	0.230	TCC	0.159	TCA	0.228	TCG	0.091
TAT	0.566	TAC	0.434	TAA	0.389	TAG	0.167
TGT	0.584	TGC	0.416	TGA	0.444	TGG	1.000

CTT	0.264	CTC	0.140	CTA	0.113	CTG	0.076
CCT	0.330	CCC	0.159	CCA	0.372	CCG	0.139
CAT	0.641	CAC	0.359	CAA	0.702	CAG	0.298
CGT	0.145	CGC	0.068	CGA	0.129	CGG	0.079
ATT	0.423	ATC	0.324	ATA	0.253	ATG	1.000
ACT	0.282	ACC	0.242	ACA	0.377	ACG	0.098
AAT	0.542	AAC	0.458	AAA	0.540	AAG	0.460
AGT	0.171	AGC	0.121	AGA	0.363	AGG	0.215
GTT	0.397	GTC	0.173	GTA	0.156	GTG	0.274
GCT	0.347	GCC	0.190	GCA	0.348	GCG	0.115
GAT	0.687	GAC	0.313	GAA	0.585	GAG	0.415
GGT	0.335	GGC	0.140	GGA	0.340	GGG	0.185

Table S3. Product testing in the p1300BR plasmid.

Original number	Test samples	Test samples	Lutein		β -Carotene		Zeaxanthin		β -cryptoxanthin		Lycopene		α -Carotene	
	Dilution volume (mL)	Sample quality (g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)
Mock p1300 BR	0.500	0.510	3333.81	3267.160	39.91	39.112	3768.07	3692.738	94.25	92.366	40.61	39.798	41.33	40.504
	0.500	0.511	193.6	189.284	4.14	4.048	6318.08	6177.239	17.66	17.266	4.53	4.429	7.57	7.401

Original number	Phytoene		Violaxanthin		Capsorubin		Capsanthin		Astaxanthin		Astaxanthin		Antheraxanthin	
	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)	Concentration (ng/mL)	Sample content (ng/g)
Mock p1300BR	8.88	8.702	1863.26	1826.009	102.37	100.323	1234.06	1209.388	724.74	710.251	648.530	635.564	455.539	446.432
	0.98	0.958	359.83	351.809	251.47	245.864	19042.59	18618.097	741.51	724.980	663.663	648.869	286.326	279.943

Table S4. List of all primers used in this study.

Name	aim of use	Sequence (5'-3')
crtF	35S:TP:CrtW (cloning site: <i>Pst</i> I+ <i>Sac</i> I)	<u>ATTCGTACGACCCTCCTGCAGTGAGACTTTTCAACAAAGGGTA</u>
crtR		<u>TTGTGATGTCACTTCGAGCTCTCATTAAAGATTCACCTCTCCA</u>
cZF	rbcs:TP:CrtZ (cloning site: <i>Pst</i> I+ <i>Sac</i> I)	<u>ATTCGTACGACCCTCCTGCAGTGTGGGAACGAGATAAGGGCG</u>
cZR		<u>TTGTGATGTCACTTCGAGCTC</u> TCATTAAAGCTCCTGAACTACT
L5F	qRT-PCR for Psy gene (GeneBank no:X68017.1)	TGGTGCAGGAGAACAGACGAACTT
L5R		AGCAGCATCGAGCATGTCAAATGG
L6F	qRT-PCR for CrtZ0 gene (GeneBank no:Y09225.1)	TCCCGTGCCATTTGTCTCCAGC
L6R		GCACAAAGCAAGCAGCCAACCT
L7F	qRT-PCR for Lcyb gene (GeneBank no:X86221.1)	AGTTGTTGGAATTGGTGGCACAGC
L7R		TTATGGCATTGGCAACGACAGGAG
L8F	qRT-PCR for Ccs gene (GeneBank no:X76165.1)	TTAGCACCCACATCAAAGCCAGAG
L8R		TGGTGAAGGGTCAACGCAACATAC
L9F	qRT-PCR for Ubi gene (GeneBank no:AY486137.1)	GAGCAGTGGAGTCCAGCATTAAACC
L9R		TTCTGAGTCCAGCTACGAGCAGTG
L10F	qRT-PCR for CrtW gene	CTCCAGGAAGGCCAAGGCTTAATG
L10R		CAGTACCAGGTGCAGCATGATGAG
L11F	qRT-PCR for CrtW gene	GTTATGGTGGCAGTGGGACTTCATC
L11R		GCCTATGTGCTTGTATACGCCTTGT