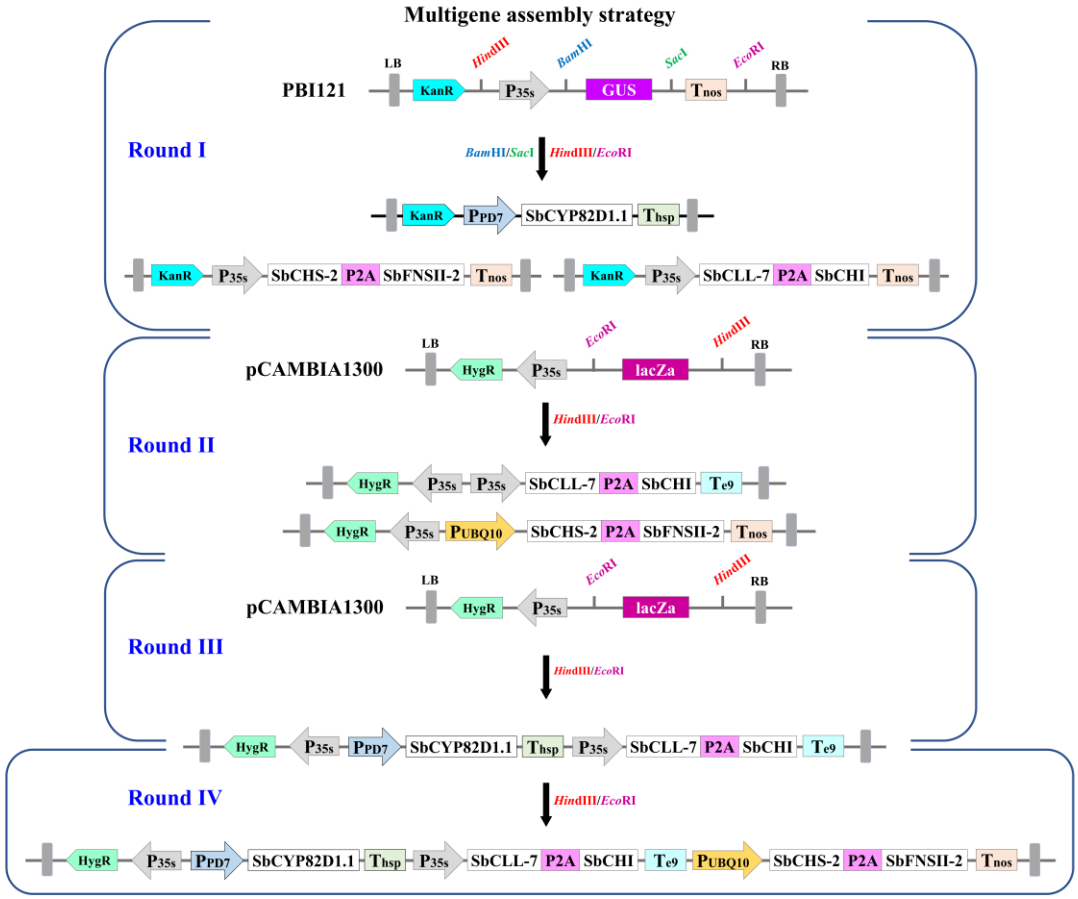

Figure S1. The multigene assembly strategy for baicalein biosynthesis genes. Total 4 rounds of multigene assembly have been performed in this experiment. The amino acid sequence of P2A is GSGATNFSLLKQAGDVEENPGP. *Bam*HI/*Sac*I and *Hin*dIII/*Eco*RI are restriction enzyme sites. HygR was hygromycin, KanR was kanamycin.

ATAGGCAACCGTGGACTTCTTCACTAGCTCATCGAGATAGCAATGCCACTAGCTAATT
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TCCTAGTTCTTAGGATGAACTGGTGTAATAAACAGCAAAAAAAAAAAAAAAAAAAA
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GGCGTGCACCGAGCCGTGGTGGTGAGCAGCTGGGAGCTAGCCAAGGAGATCTTCACC
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TATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGC
GCGCGGTGTCATCTATGTTACTAGATC

Figure S2. The nucleotide sequence of insertion fragment in the multigene vector FCC-CF. The baicalein biosynthesis genes *SbCYP82D1.1* (blue), *SbCLL-7* (pink), *SbCHS-2* (black), *SbCHI* (purple) and *SbFNSII-2* (gray) were showed in this graph. P2A peptides sequences was highlighted in red. The nucleotide sequences of two 2A peptides was different, but there was the same amino acid sequence of GSGATNFSLLKQAGDVEENPGP. *SbCYP82D1.1* gene was controlled by *AtPD7* promoter (red), and the terminator was heat-shock protein 18.2 terminator (Thsp) (highlighted in light yellow). *SbCLL-7*-2A-*SbCHS-2* was *CaMV 35S* promoter (green) and terminator from pea rbcS-E9 gene (Te9) (highlighted in light green). *SbCHI*-2A-*SbFNSII-2* was under the control of *AtUBQ10* promoter (orange) and terminator of nopaline synthase gene (Tnos) (highlighted in light blue).

Table S1. Primers used for multigene assembly

Primers for Round I of multigene assembly

Primer	Sequences (5' to 3')
PBI121-PD7-F	tatgaccatgattacgccaagcttATAGGCAACCGTGGACTTCTTC
PBI121-PD7-R	gctcaactccatTTGAGGCTAGGTTTTAGTAGTGAAG
PD7-SbCYP2D1.1-F	agcctcaaATGGAGTTGAGCTCTGTCATCTATG
PD7-SbCYP2D1.1-R	tcttcatcttcatatTAATATAGAGTAGGTGACAACCTTGG
SbCYP2D1.1-Thsp-F	aatcgtttaATATGAAGATGAAGATGAAATATTTG
SbCYP2D1.1Thsp-R	aaaacgacggccagtgaattcCTTATCTTTAATCATATTCCATA
PBI121-SbCLL-7-F	acgggggactctagaggatccATGGAGAAATCGGGCTATGG
	tgCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCCTAACTTCGATCGGACCT
PBI121-SbCLL-7-R	TTTC
	aacttcagcctgctgaagCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGTC
PBI121-SbCHI-F	TGCTTCGCCATCCGT
PBI121-SbCHI-R	cgatcggggaaattcgagctcTTAAAACAACTCCGATAGTCTTGC
PBI121-SbCHS-2-F	acgggggactctagaggatccATGGTGACAGTTGAAGAATTCCAC
	ggcctgTTTCAAGAGAGAAAAATTAGTGGCACCTGAACCATTGAGAGGCACA
PBI121-SbCHS-2-R	CTATGC
	cttgaaaCAGGCCGGTGACGTTGAAGAGAACCCAGGTCCAATGGAAGTCACA
PBI121-SbFNSII-2-F	CTGAATGTGG
PBI121-SbFNSII-2-R	cgatcggggaaattcgagctcTCAGTGCCCGGAAATAACCC

[a] The homology arm sequences are noted in red; All restriction enzyme sites are underlined.

[b] The sequence of P2A polypeptides are noted in green.

Primers for Round II of multigene assembly

Primer	Sequences (5' to 3')
1300-35S-F1	ctatgacatgattacgaattcTGAGACTTTTCAACAAAGGGT
1300-35S-R1	gcccgatttctccatTGTTCTCTCCAAATGAAATGAACTTC
35S-CLL-CHI-F1	tggagagaacaATGGAGAAATCGGGCTATGGC
35S-CLL-CHI-R1	gaacgaaagctTTAAAACAACTCCGATAGTCTTGCC
1300-Te9-F1	ttgttttaaAGCTTTCGTTCGTATCATCG
1300-Te9-R1	acgacggccagtgccaagcttTTGATGCATGTTGTCAATCAAT
1300-UBQ-F1	ctatgacatgattacgaattcGTCGACGAGTCAGTAATAAACGGC
1300-UBQ-R1	cttcaactgtcaccatCTGTTAATCAGAAAAACTCAGATTAATCG
35S-CHS-FNS-F1	ctgattaacagATGGTGACAGTTGAAGAATTCCACC
35S-CHS-FNS-R1	tttgaacgatcTCAGTGCCCGGAAATAACCC
1300-Tnos-F1	gggcactgaGATCGTTCAAACATTTGGCAATAA
1300-Tnos-R1	acgacggccagtgccaagcttGATCTAGTAACATAGATGACAC

[a] The homology arm sequences are noted in red; All restriction enzyme sites are underlined.

Primers for Round III of multigene assembly

Primer	Sequences (5' to 3')
PD7-SbCYP2D1.1-Thsp-F	ctatgacatgattacgaattcATAGGCAACCGTGGACTTCTTC
PD7-SbCYP2D1.1Thsp-R	cctttgttgaaaagtctcaCTTATCTTTAATCATATTCCATAGTCCATACC
35S-CLL-CHI-Te9-F2	ttaaagataaTGAGACTTTTCAACAAAGGGTAATATC
35S-CLL-CHI-Te9-R2	acgacggccagtgccaagcttTTGATGCATGTTGTCAATCAAT

[a] The homology arm sequences are noted in red; All restriction enzyme sites are underlined.

Primers for Round IV of multigene assembly

Primer	Sequences (5' to 3')
FCC-F3	ctatgacatgattacgaattcATAGGCAACCGTGGACTTCTTC
FCC-R3	ttactgactcgtcgacCTTGGACTCCCATGTTGGCA
CF-F3	aacatgggagtccaagGTCGACGAGTCAGTAATAAACGGC
CF-R3	acgacggccagtgccaagcttGATCTAGTAACATAGATGACAC

[a] The homology arm sequences are noted in red; All restriction enzyme sites are underlined.

Table S2. Primers for PCR detection

Primer	Sequences (5' to 3')
SbCYP82D1.1-F	ATGGAGTTGAGCTCTGTCATCTATG
SbCYP82D1.1-R	TAATATAGAGTAGGTGACAACCTTGG
SbCLL-7-F	ATGGAGAAATCGGGCTATGG
SbCLL-7-R	TTATAACTTCGATCGGACCTTTTC
SbCHI-F	ATGTCTGCTTCGCCATCCGT
SbCHI-R	TTAAAACAACTCCGATAGTCTTGC
SbCHS-2-F	ATGGTGACAGTTGAAGAATTCCAC
SbCHS-2-R	TCAATTGAGAGGCACACTATGC
SbFNSII-2-F	ATGGAAGTCACACTGAATGTGG
SbFNSII-2-R	TCAGTGCCCGGAAATAACCC
Hyg-F	TTGGCGACCTCGTATTGGGA
Hyg-R	CAAGACCTGCCTGAAACCGAA

Table S3. Primers for qRT-PCR analysis

Primer	Sequences (5' to 3')
SbCYP82D1.1-qF	TTGACAAAAAGGTGGAGGTT
SbCYP82D1.1-qR	CAACACTCTCGGTCATATCC
SbCLL-7-qF	ATTGAGGCCACCGTTGTATC
SbCLL-7-qR	CGACTTTTGAAACCGTGGAT
SbCHI-qF	AAGGCAGTAATAGAGAACAAACAG
SbCHI-qR	TTAAAACAACTCCGATAGTCTTG
SbCHS-2-qF	TTCGTGATGGATGAGATGAG
SbCHS-2-qR	CCATTGAGAGGCACACTATG
SbFNSII-2-qF	TCACCTATGGCGTCTCCTTC
SbFNSII-2-qR	CAGCTCCTCAGTGACGTTGA
Leactin-qF	CCAGGTATTGCTGATAGAATGAG
Leactin-qR	GAGCCTCCAATCCAGACAC