

Correlations between *SmCPS1* Promoter Polymorphism and Tanshinone Contents in *Salvia miltiorrhiza*

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Electronic Supplementary Material (ESM)

Promoter isolation

Seven pairs of primers used were in Table S6. These primers were designed using KF718290.2 (genbank accession number) and the scaffold sequence of *S. miltiorrhiza* as references.

PCR reactions were conducted in a 50 µl volume consisting of 35–60 ng template DNA, 5.0 µl of 10× PCR buffer, 4.0 µl of MgCl₂ (25 mM), 4.0 µl of dNTP Mixture (2.5 mM), 2 µl of each primer (10 µM), 1 U Taq.

PCR conditions for all primer pairs except for P3 were as follows: initial denaturation at 94°C for 3 min followed by 45 cycles of amplification; each cycle consisted of 94°C for 30 s, Ta (Table 2) for 30 s and elongation at 72°C for 70 s. Then a final extension at 72°C for 5 min was adopted to complete the reaction. A touchdown PCR for primer pair P3 was as follows: initial denaturation at 94°C for 3 min followed by 45 cycles of amplification; 94°C for 30 s, Ta (dropping by 0.2 per cycle to 45°C) for 30 s and 72°C for 70 s for the first 35 cycles; then 94°C for 30 s, 45°C for 30 s and 72°C for 70 s for ten cycles. Then the reaction was completed after a final extension at 72°C for 5 min.

The assembled sequences were aligned and truncated to the same ends for further analysis.

Table S6. Primer pairs used in the amplification of *SmCPS1* promoter and 388 bp downstream translation start site

Primer	Sequence(5'–3')	Ta (°C)	Amplicon (bp)"
P1	F: ACTCAAATCCTAAACCCGTTCC R: CATCTACTGTGGCAACTGTGAA	56	606
P2	F: AGGCAGAAAAAAGAAAAACATGATT R: TGAGACAAGATCGTGGGATAAA	52	1073
P3	F: ACGGTTATTAAGGAGAATATTATTAGTG R: TGAGACAAGATCGTGGGATAAA	52	785
P4	F: AAATAGGCAGGGAAATGTTAATTAATTA R: CATTTCACATCCAATTAACCTCAAAAC	52	524
P5	F: GATTACTAGTGCAATAAATGAGATG R: CACCTTTCATCTCCTACTTTATCAA	50	830
P6	F: AAGGTGGCGGTAGTCAA R: CTCCTTAATAACCGTGCAGAAAC	53	1027
P7	F: GAGAGTTGTCGATTAACTACTAC R: TGTATGGATACAACCTGAACACCTC	52	765

Ta – annealing temperature