1 **Efficient synthesis of eriodictyol from L-tyrosine in *Escherichia coli***

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5 **Running title: Efficient synthesis of eriodictyol in *Escherichia coli***

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21 **Materials and methods**

22 **RNA preparation and qPCR**

23 Total RNA of strains (BF1, BF2, BMF1 and BMF2) was purified using the
24 RNAprep Pure Cell/Bacteria Kit (TIANGEN, Beijing, China). *E.coli* total RNA was
25 reverse transcribed with the PrimeScript RT reagent (TaKaRa, Dalian, China).
26 Quantitative reverse transcription-PCR (qPCR) was used to measure the mRNA levels
27 of specific genes in different plasmids, done by the LightCycler 480 II Real-Time
28 PCR System (Roche Diagnostics, Mannheim, Germany) and SYBR Premix Ex Taq
29 kits (TaKaRa, Dalian, China). The designed primers were showed in Table S1. 16S
30 rRNA reverse transcriptase-PCR products were used as internal standards (1).

31 It was confirmed that the combination of pCDF-*TAL-4CL* and pET-*CHS-CHI*
32 could prevent the accumulation of coumaroyl-CoA and reverse *TAL* inhibition in our
33 previous works (2, 3). We also performed the qPCR experiment to make the process
34 description more reliable. Another two strains were constructed: BQ1
35 (pCDF-*TAL-4CL* and pET-*CHS-CHI*) and BQ2 (pET-*TAL-4CL* and pCDF-*CHS-CHI*).

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37 **Table S1. Oligonucleotides used in qPCR**

Name	Oligo Sequence (5'-3')	Target gene
q16S-F	CTTCTTCTGCGGGTAACG	16S rRNA
q16S-R	CACTGGAACTGAGACACG	16S rRNA
q*TAL*-F	GAGGTTAAGGTTAAGGATGATG	*TAL*
q*TAL*-R	TGATGGCTGGTCTTGTTC	*TAL*

q*4CL*-F	CTGCTGTCCCGTAAAGTTG	*4CL*
q*4CL*-R	CGCCATAGTGCTGATTGC	*4CL*
q*CHS*-F	ATGGTTACGGTGGAAGAATAC	*CHS*
q*CHS*-R	ATGTTCAGAGTTGGTGATACG	*CHS*
q*CHI*-F	GGTCACGGGCAAATCATAC	*CHI*
q*CHI*-R	TCCAGCAGTTCTTCAGAGC	*CHI*
q*acs*-F	AGATGGCTATTACTGGATAAC	*acs*
q*acs*-R	TGCGGAATACCTACTACG	*acs*
q*ACC*-F	GAAGAAGGCGGCGTTGAAG	*ACC*
q*ACC*-R	TCGTCGGTGAGGCATTCG	*ACC*
q*tFC*-F	TTATTCCATCAGTTCCTCAC	*tF3'H-CPR*
q*tFC*-R	ATTGCGTTCTTCATCCAG	*tF3'H-CPR*

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39 **Results**

40 **Transcriptional levels of genes on different vectors**

41 The results of qPCR were showed in Table S2. The qPCR results showed that the
42 transcriptional levels of *ACC* in BMF2 was 7.93 times as that of BMF1. The
43 transcriptional levels of *acs* in BMF2 was 8.19 times as that of BMF1. The
44 transcriptional levels of *tF3'H-tCPR* in BF2 was 0.13 times as that of BF1. The
45 transcriptional levels of *tF3'H-tCPR* in BMF2 was 0.12 times as that of BMF1. The
46 transcriptional levels of *TAL* in BQ2 was 2.31 times as that of BQ1. The
47 transcriptional levels of *4CL* in BQ2 was 2.11 times as that of BQ1. The

48 transcriptional levels of *CHI* in BQ2 was 0.65 times as that of BQ1. The
49 transcriptional levels of *CHS* in BQ2 was 0.47 times as that of BQ1.

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51 **Table S2. The transcriptional levels of genes in different strains.**

Genes	The fold-change of transcriptional levels
ACC (BMF2/BMF1)	7.93±0.22
acs (BMF2/BMF1)	8.19±0.23
tF3'H-tCPR (BF2/BF1)	0.13±0.01
tF3'H-tCPR (BMF2/BMF1)	0.12±0.01
TAL (BQ2/BQ1)	2.31±0.07
4CL (BQ2/BQ1)	2.11±0.05
CHI (BQ2/BQ1)	0.65±0.04
CHS (BQ2/BQ1)	0.47±0.03

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