frontiers

SUPPLEMENTAL MATERIAL

Construction of an artificial biosynthetic pathway for the styrylpyrone compound 11-methoxy-bisnoryangonin, produced in engineered *Escherichia coli*

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Table S1. ^{13}C (175 MHz) and ^{1}H (700 MHz) NMR spectroscopic data for 11-methoxy-bisnoryangonin.

Figure S1. Selected mass ion chromatogram and the proposed structures of each chalcone peak (denoted by an asterisk) from analyses of reaction products shown in Figure 5. A) Peak at 12.7 min (trace (a) in Figure 5) exhibited *m/z* 257 $[M+H]^+$ and *m/z* 255 $[M-H]^-$, which corresponded to a chalcone compound (MW 256). B) Peak at 9.8 min (trace (b) in Figure 5) exhibited *m/z* 273 $[M+H]^+$ and *m/z* 271 $[M-H]^-$, which corresponded to a chalcone compound (MW 272). C) Peak at 8.5 min (trace (c) in Figure 5) exhibited *m/z* 289 $[M+H]^+$ and *m/z* 287 $[M-H]^-$, which corresponded to a chalcone compound (MW 288). D) Peak at 11.0 min (trace (b) in Figure 5) exhibited *m/z* 311 $[M+H]^+$ and *m/z* 309 $[M-H]^-$, which corresponded to 4-coumarate dehydrodimer (MW 310).

Figure S2. HPLC profile and the proposed structures of *in vitro* enzymatic reactions with cinnamic acid (A), 4-coumaric acid (B), and caffeic acid (C). Lower panels represent standard phenylpropanoic acids and upper panels represent reaction results. The absorbance was monitored at 280 nm. Cinnamic acid, 4-coumaric acid, and caffeic acid formed new peaks (12.7 min, 9.8 min, and 8.5 min, respectively) corresponding to the molecular weights of different chalcone compounds, which are denoted by asterisks.

Figure S3. LC/MS/MS analysis of dimer compound. (A) The peak at 10.9 min exhibited parent mass ion peaks at *m/z* 371 $[M + H]^+$ and *m/z* 369 $[M - H]^-$, which corresponded to molecular weight 370 Da. (B) Structure and MS/MS spectra of the predicted dimer compound.

Figure S4. Schematic illustration showing the strategies for constructing the genome engineered strain.

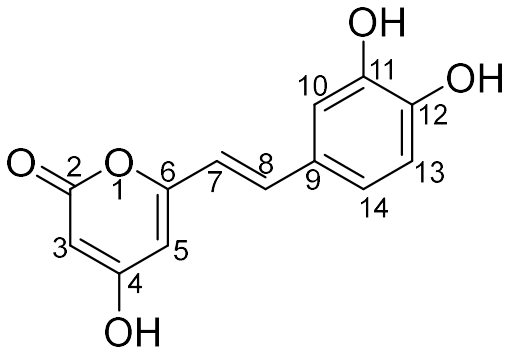
A) Construction of the L-tyrosine overproducing strain of *E. coli* (ΔCOS1) was achieved by extra gene insertion of *aroG* and *tyrA*, feedback-inhibition resistance (fbr) genes on the *tyrR* gene locus in C41(DE3) strain (Kang *et al.* 2015). B) Construction of the ferulic acid overproducing strain of *E. coli* (COS6-T5M) was achieved by extra gene insertion of T5M module (*optal*, *sam5*, and *com* gene) on the *bioC* gene locus in ΔCOS1 strain (Kang *et al.* 2018).

Table S1 . *13 C (175 MHz) and 1H (700 MHz) NMR Spectroscopic Data for 11-methoxyl-bisnoryangonin..*

	hispdin[a]		11-methoxyl-bisnoryangonin[b]	
Position	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)
2	168.82		170.4	
3	89.50	5.23(1H, s)	89.3	5.30(1H, d, 2.0)
4	169.85		159.9	
5	101.30	6.13(1H, s)	100.5	6.13(1H, d, 2.0)
6	160.35		163.1	
7	116.91	6.67(1H, d, 16)	116.7	6.84(1H, d, 16.0)
8	134.81	7.12(1H, d, 16)	134.7	7.22(1H, d, 16.0)
9	127.26		126.8	
10	114.47	7.03(1H, d, 2.0)	110.7	7.27(1H1 d, 1.9)
11	145.95		148.4	
12	147.88		148.0	
13	116.16	6.77(1H, d, 8.1)	115.7	6.79(1H, d, 8.1)
14	120.73	6.95(1H, dd, 8.1;2.0)	122.0	7.07(1H, dd, 8.2,;1.8)
10-OCH3			55.7	3.82(3H, s)

[a]Li-feng ZAN et al. (2011) for the literature values of ^{1}H and ^{13}C NMR data

[b]11-methoxyl-bisnoryangonin in DMSO-d_6 (700MHz for ^{1}H and 175MHz for ^{13}C NMR data)



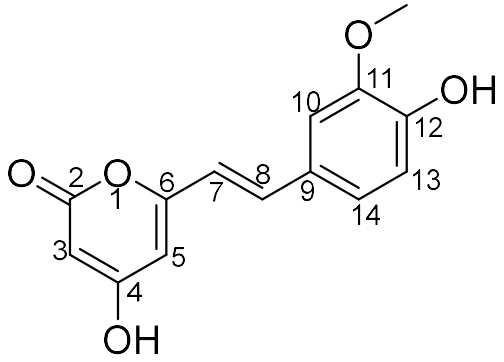


Figure S1. Selected mass ion chromatogram and the proposed structures of each chalcone peak (denoted by an asterisk) from analyses of reaction products shown in Figure 5.

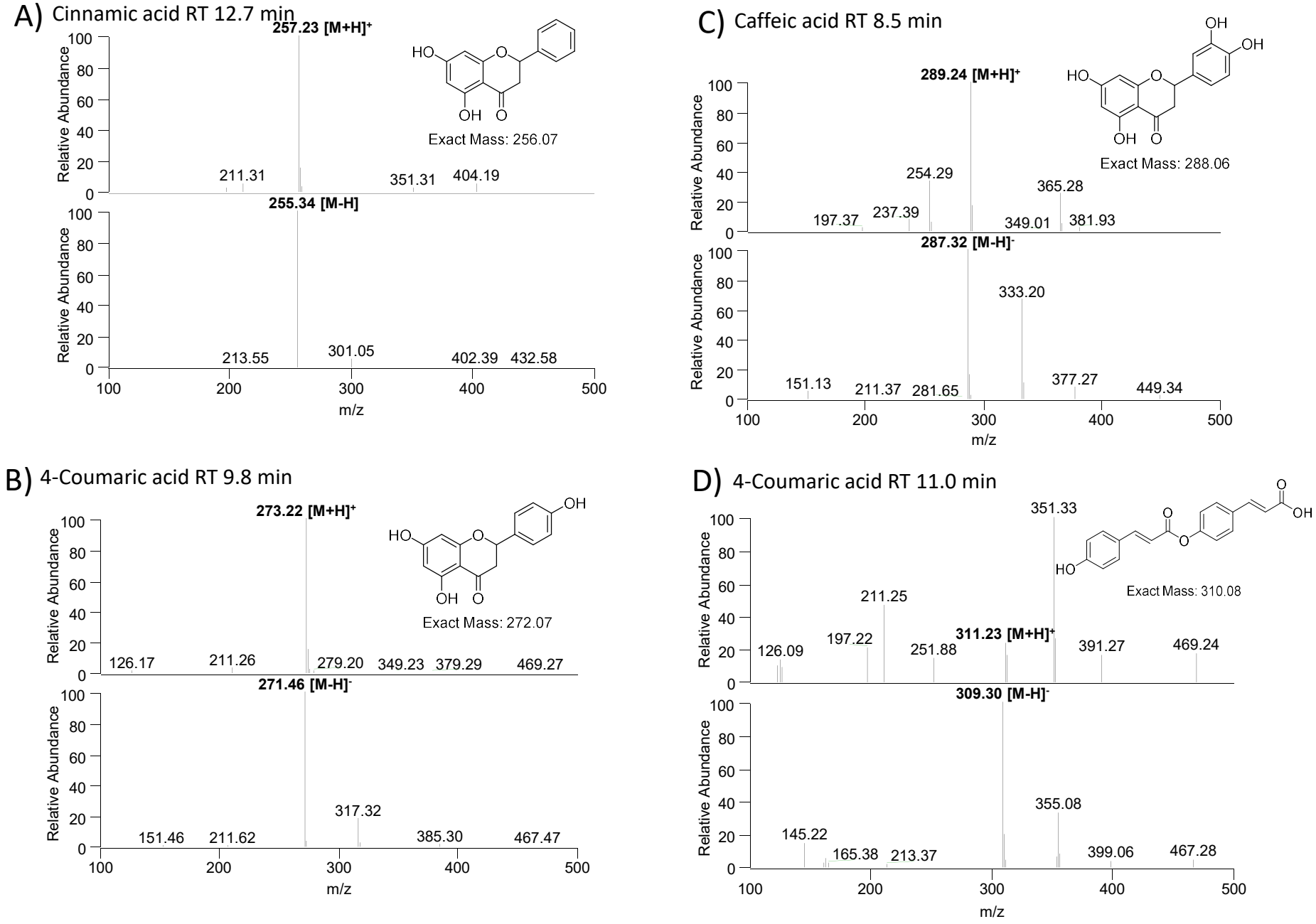


Figure S2. HPLC profile of *in vitro* enzymatic reactions with cinnamic acid (A), 4-coumaric acid (B), and caffeic acid (C).

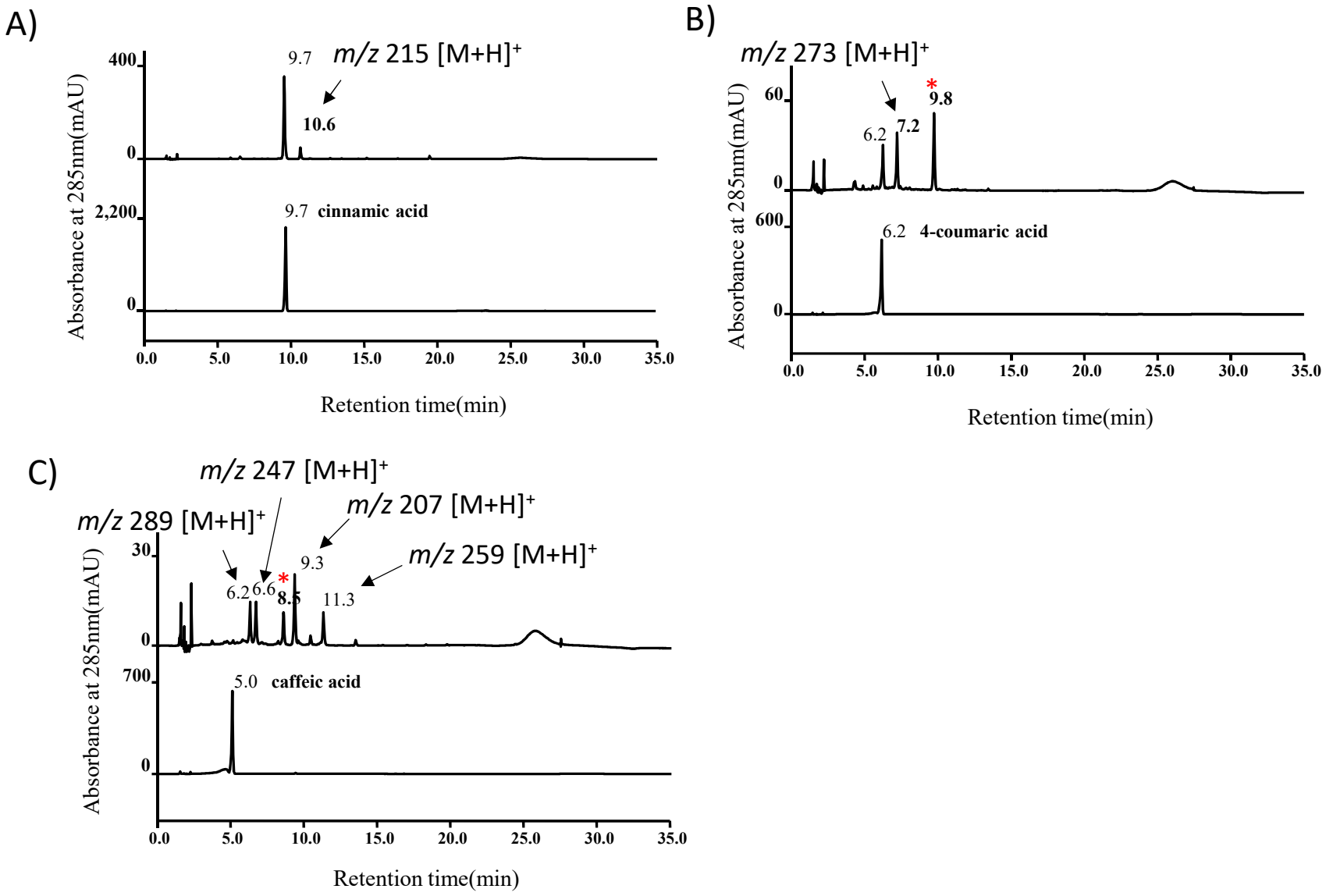


Figure S3. LC/MS/MS analysis of dimer compound.

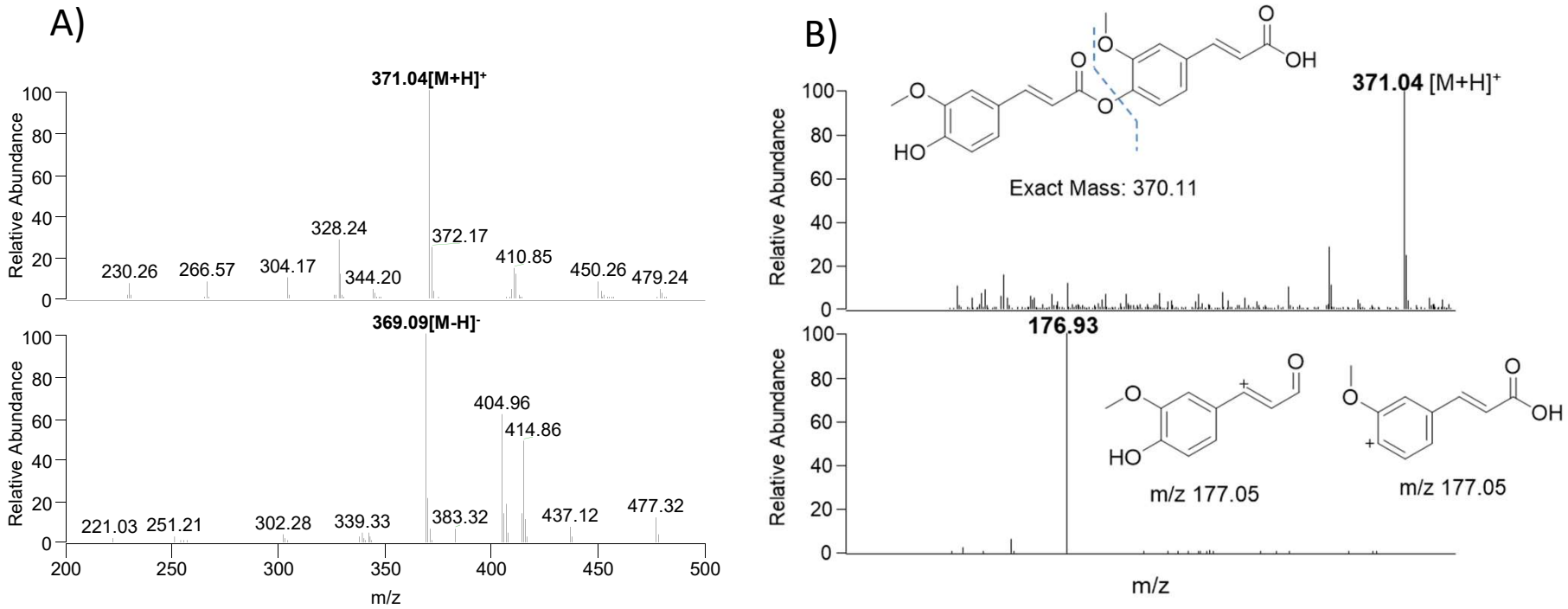
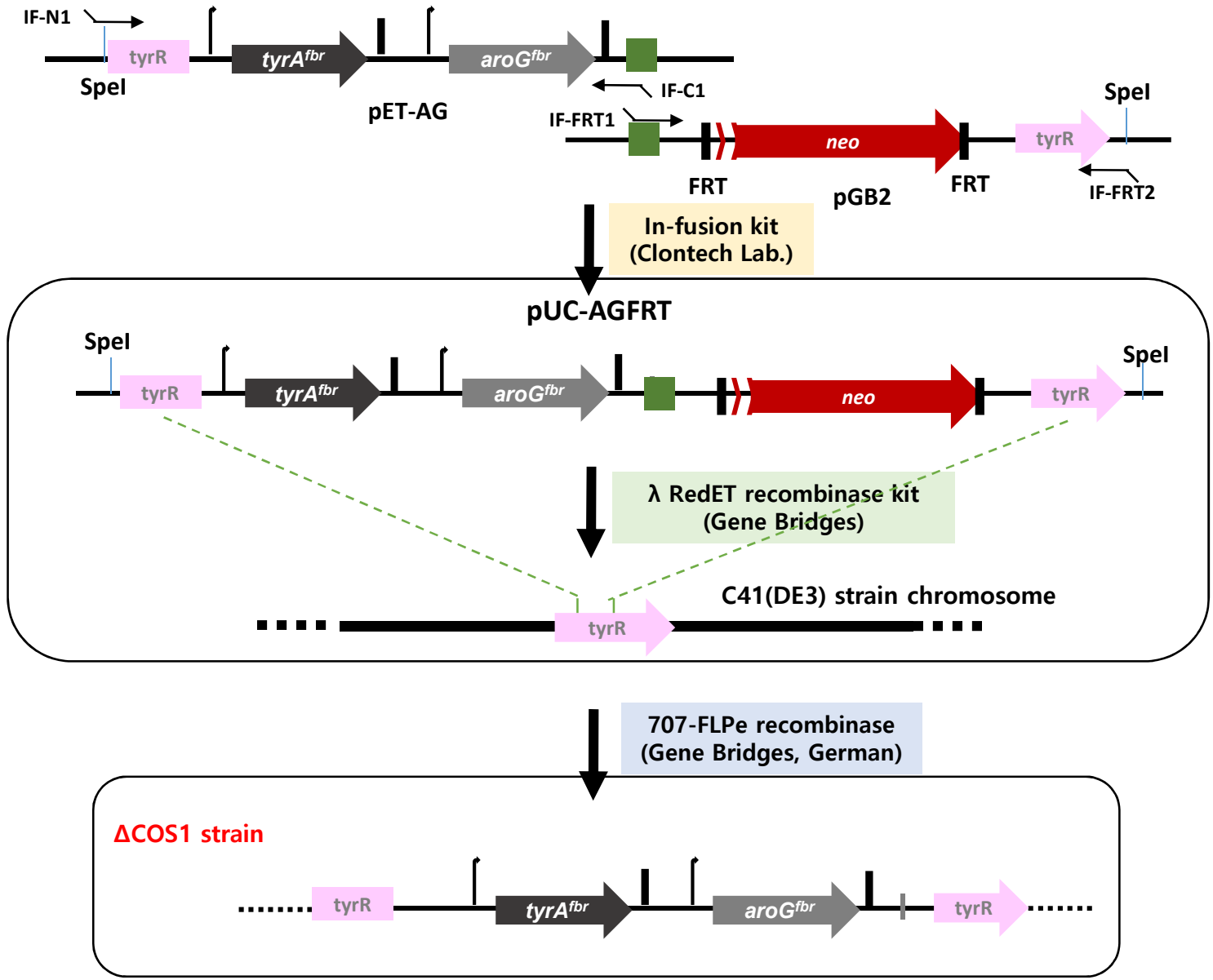
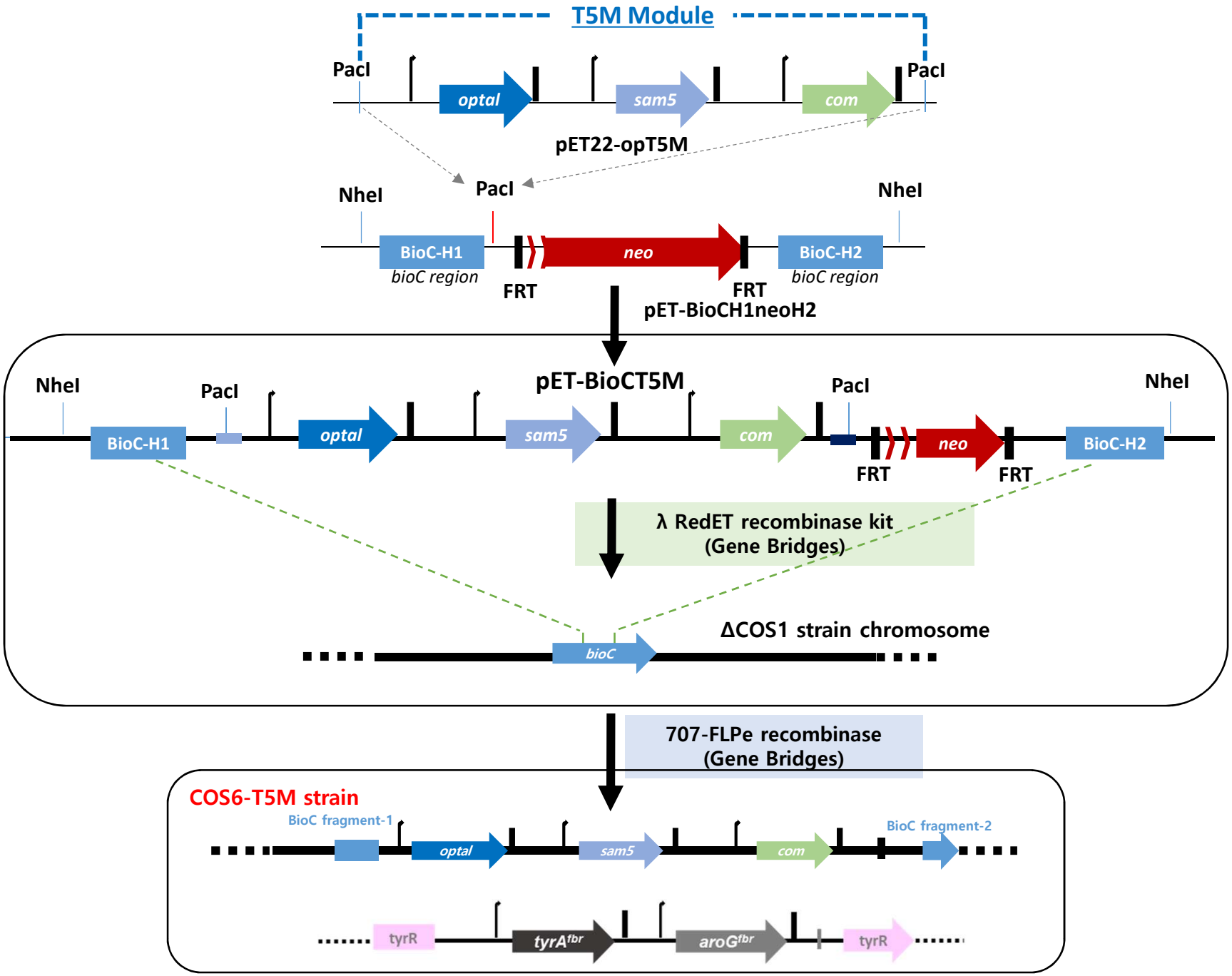


Figure S4. Schematic illustration showing the strategies for constructing the genome engineered strain.

A) Construction of the L-tyrosine overproducing strain of *E. coli* (ΔCOS1)



B) Construction of the ferulic acid overproducing strain of *E. coli* (COS6-T5M)



>pnPKS *Piper nigrum* styrylpyrone synthase cDNA

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