

Supplementary materials

Heterologous biosynthesis of prenylated resveratrol through multiplex metabolic
engineering in *Escherichia coli*

Haijiao Wang^{a,b,c,#}, Ting Zhou^{a,b,c,#}, Hui Liu^{a,b,c}, Lingrong Wen^{a,b,c}, Yueming Jiang^{a,b,c},

Bao Yang^{a,b,c,*}

^a Guangdong Provincial Key Laboratory of Applied Botany, Key State Laboratory of
Plant Diversity and Specialty Crops, Key Laboratory of National Forestry and
Grassland Administration on Plant Conservation and Utilization in Southern China,
South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650,
China

^b South China National Botanical Garden, Guangzhou 510650, China

^c University of Chinese Academy of Sciences, Beijing 100049, China

Table S1 Primers used in this study.

| Name | Seuqence | Description |
|--------------|---|---|
| pJ-F1 | ATGAGCGAATTACGTGCATTGAGC | Primers used to amplify the fragments for pJBEI6410-1 |
| pJ-R1 | GTATATCTCCTTCTTAAAAGATCCTTAA TTCTGACGAAATG | |
| pJ-F2 | GACAGTAATTAAGCCGCCATTGAACG | |
| pJ-R2 | ACGTAATTCGCTCATATGATCTCCCT | |
| pJ-F3 | GCTTTAATTACTGTGCCCCCAGG | |
| pJ-R3 | AAGAAGGAGATATACGGATCCAAACT CGAGTAAGGATCTCC | |
| pJ-F4 | CACAACGGCACGATCAACACG | Primers used to amplify the fragments for pJBEI6410-2 and pJBEI6410-3 |
| pJ-R4 | CTCGAGTTTGGATCCGTATATCTCCTTC TAAAAGATCCTGAAATTGT | |
| pJ-F5 | GTCCCGGTAAATGTTGTCACCTCG | |
| pJ-R5 | GATCGTGCCGTTGTGGCTGTCTTTC | |
| pJ-F6 | GGATCCAAACTCGAGTAAGGATCTCC | |
| pJ-R6 | AACATTTACGGGACGATTTCCG | |
| pJ-R7 | GTATATCTCCTTCTTAAAAGATCCTGA AATTGT | |
| IacE-F2 | AAGAAGGAGATATACATGGCTATCTCA ACCCCTTCCAAC | Primers used to insert At4CL and VvSTS into pCDFDuet-1 for pCDFRESa. |
| IacE-R2 | CTCGAGTTTGGATCCTTAGTAGTAGAA CTCG | |
| 4CL-BamHI-F | ACCATCATCACCACAGCCAGGATCCGA TGCGCCGCAGGAACAG | |
| 4CL-NotI-R | GTTGACTTAAGCATTATGCGGCCGCC AGACCGTTCGCCAGTTTCG | Primers used for the fusion of At4CL-VvSTS, used with 4CL-BamHI-F and STS-XhoI-R to construct pCDFRESf. |
| STS-NdeI-F | GTATAAGAAGGAGATATACATATGGCG TCTGTTGAAGAA | |
| STS-XhoI-R | GCAGCGGTTTCTTTACCAGATTAGTTG GTAACGGTCGGA | |
| 4CL-fusion-R | TTTCGCCGCCGCTTCCAGACCGTTCGCC AGTTTCG | Primers for anti-sense RNA expressing plasmids |
| STS-fusion-F | GAAGCGGCGGCGAAAATGGCGTCTGTT GAAGAA | |
| Plac-pET28-F | TGCGTCCGGCGTAGAGGATCGTCTAGA GACTGGAAAGCGGGCAGTG | Primers for anti-sense RNA expressing plasmids |
| Plac-R | CCTCCATGGTGAAATTGTTATCCGCTC ACAATTCC | |
| asgltA-lac-F | CGGATAACAATTTACCATGGAGGAGG AATTAACCATGCAGTGGTGGTGGTGGT | |

| | | |
|---------------|--|--|
| | GGTGCACATCCAGTTCAACAGCTG | |
| asgltA-R | GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGG GTTTTCCCCTCTTTCACAG | |
| lac-asacpS-F | GATAACAATTTACCATGGAGGAGGAA TTAACCATGCAGTGGTGGTGGTGGTGG TGCATTTCGTTATCGCTTAATACGC | |
| asacpS-R | GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGG CATCCCTGAGATGCATG | |
| lac-asacpT-F | GGATAACAATTTACCATGGAGGAGGA ATTAACCATGCAGTGGTGGTGGTGGTGG GTGATAGATGATCTCCGGTAGCG | |
| ascapT-R | GCTGTCCACCAGTCATAGGAGGAATTA ACCATGCAGTGGTGGTGGTGGTGGTGG AAGGCGGTAGCAAAGT | |
| Target-accA-F | GTAAGTGGAGTTTACTAATACGTTTT AGAGCTAGAAATAGCAAGTT | |
| Target-accA-R | GTATTAGTCAAACCTCCAGTTACTAGTA TTATACCTAGGACTGAGC | |
| Target-accB-F | ATCACACTAAACAAAGAGTAGTTTTAG AGCTAGAAATAGCAAGTT | Primers used for sgRNA expressing plasmids pTargetF-accA, pTargetF- accB, and pTargetF-ACS. |
| Target-accB-R | TACTCTTTGTTTAGTGTGATACTAGTAT TATACCTAGGACTGAGC | |
| Target-acs-F | CATATTATTAACATCCTACAGTTTTAGA GCTAGAAATAGCAAGTT | |
| Target-acs-R | CTGTAGGATGTTAATAATATGACTAGT ATTATACCTAGGACTGAGC | |
| 119-C-F | TTGACAGCTAGCTCAGTCCTAGGTATA ATGCTAGCTAATAGAAATAATTTTGTT TAACT | For amplification of J23119 promoter with an artificial RBS and a 6His tag. |
| 119-C-R | TGATGCATTCTTGCCTCTTAACTTTAAA GTTAAACAAAATTATTCTATTAGCTA GCAT | |
| accA-119-Dn-F | TAAGAGGCAAGAATGCATCATCACCAT CACCACATGAGTCTGAATTTCTTGATT TTGA | For amplification of <i>accA</i> homologous arms |
| accA-119-Dn-R | GACGATAGCTTTATCGTCTGC | |
| accA-119-UP-F | GCTTGCTATCTCGCTGAC | |
| accA-119-UP-R | GGACTGAGCTAGCTGTCAACACCGCCA GAGATAATTAGTC | |
| 119-accB-Dn-F | TAAGAGGCAAGAATGCATCATCACCAT CACCACATGAGTCTGAATTTCTTGATT TTGA | For amplification of <i>accB</i> homologous arms |

| | | |
|---------------|---|--|
| 119-accB-Dn-R | GACGATAGCTTTATCGTCTGC | |
| 119-accB-UP-F | GCTTGCTATCTCGCTGAC | |
| 119-accB-UP-R | GGACTGAGCTAGCTGTCAACACCGCCA GAGATAATTAGTC | |
| 119-acs-Dn-F | TAAGAGGCAAGAATGCATCATCACCAT CACCACATGAGCCAAATTCACAAACAC A | For amplification of <i>ACS</i> homologous arms |
| 119-acs-Dn-R | GCTGATATGTTTGCTCTGGC | |
| 119-acs-UP-F | CACGACAGTAACCGCACC | |
| 119-acs-UP-R | GGACTGAGCTAGCTGTCAATGGCATAA GCGTTAAATGTAGG | |

Table S2 DNA sequences

| Name | Seuquence | Description |
|------------------------|---|---|
| <i>lac</i> promoter | <u>TCTAG</u> Agactggaaagcgggcagtgagcgcaacgcaattaat gtgagttagctcactcattagccacccaggctttacactttatgcttcc ggctcgtatgttgtgtggaattgtgagcggataacaattca CCAT GG | Amplified from pUC19 with XbaI/NcoI added to either ends. |
| asgltA | <u>aggaggaattaac</u> catgcagtggtggtggtggtg CACATC CAGTTCAACAGCTGTGTCCCCGTTGAG GGTGAGTTTGTCTTTGTATCAGCCATT TAAGGTCTCCTTAGCGCCTTATTGCGTA AGACTGCCGGA ACTTAAATTTGCCTTCG CACATCAACCTGGCTTTACCCGTTTGT ATTTGGCTCGCCGCTCTGTGAAAGAGG GGAAAACC <u>caccaccaccaccactgcatggttaattcct</u> <u>cct</u> | Anti-sense RNA for <i>gltA</i> . Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences. |
| asacpS | <u>aggaggaattaac</u> catgcagtggtggtggtggtg CATT CG TTATCGCTTAATACGCGGCGTGCCAGG CGATCACCGGATCGGGCGATCACCGCT TCGATGCGAGCGATCTCCACAATATCC GTGCCTAAACCTAATATTGCCATTAGCC ACGCGCTTCCAGCATCAGACGCTTCATT TCTGCCACCGCATCTTTCAGTCCGGTCA TCACTGCACGACCAATAATGGCATGAC CGATATTCAGTTCATGCATCTCAGGGAT GG <u>caccaccaccaccactgcatggttaattcctcct</u> | Anti-sense RNA for <i>acpS</i> . Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences. |
| asacpT | <u>aggaggaattaac</u> catgcagtggtggtggtggtg ATAGAT GATCTCCGGTAGCGGGAAAGCGTGTG CGAAAGCAATGCACGCCCCGCCAGCCA GCGTTCGCGTCGTGGACCTTGCGGTGC TTGCTCGCGTAAACCCGGTGGCAGTGG AGCTGCGCTTAAGGTGCAA ACTTTCCCC AGA ACTATCCGATACATATCAGGGCCA ACGTTTAATGGAAAATGAAAGTGC GTAT CGTATCACTTGTGCGCTCATCCCGGTAA CCGACTTTTCGGTCTGCCCGGCCCCAG TAAAATCGCCAGTTTGCTACCGCCTTT <u>ca</u> <u>ccaccaccaccaccactgcatggttaattcctcct</u> | Anti-sense RNA for <i>acpS</i> . Underlined letters are post trascription reverse complementary structures, bolded letters are target mRNA complementary sequences. |
| J23119 promoter region | <u>ttgacagctagctcagtcctaggtataatgctagc</u> <i>TAATAGAAA</i> <i>TAATTTTGTTAACTTTAAAGTTAAGAGGCAA</i> GAATGCATCATCACCATCACCAC | Underlined letters are J23119 promoter sequence, italic letters are artificial RBS sequence, bolded letters are His tag sequence. |
| sgRNA for <i>accA</i> | AACTGGAGTTTGACTAATAC | |

| | | |
|---|---|--|
| sgRNA for <i>accB</i> | ATCACACTAAACAAAGAGTA | |
| sgRNA for <i>acs</i> | CATATTATTAACATCCTACA | |
| DNA fragment used for <i>accA</i> promoter editing | <p><u>GCTTGCTATCTCGCTGACGGACAGGCAAA</u> <u>TTGATGACCAGCTTTTAAACCGACTCCGTC</u> <u>AGTCTCTGGAACCCACCGCTCTGGGACA</u> <u>ATCCAGTACATCTCTACTATCAGAGGGC</u> <u>GGATGCACGCGCGCGGTTGCGTTTTGGCG</u> <u>CGACGTGGCGTGTCTCTCCGAGCGATCGT</u> <u>TTATTAACGATCTCCGTGGCCTCATTGGT</u> <u>TCGGAGCAGGTGGAAGTGGAGTTTGACTA</u> <u>ATTATCTCTTTGACAGCTAGCTCAGTCCTAG</u> <i>GTATAATGCTAGCTAATAGAAATAATTTTGTTT</i> <i>AACTTTAAAGTTAAGAGGCAAGAATGCATCAT</i> <u>CACCATCACCATGAGTCTGAATTTCC</u> <u>TGATTTTGAACAGCCGATTGCAGAGCT</u> <u>GGAAGCGAAAATCGATTCTCTGACTGC</u> <u>GGTTAGCCGTCAGGATGAGAACTGGA</u> <u>TATTAACATCGATGAAGAAGTGCATCGT</u> <u>CTGCGTGAAAAAAGCGTAGAACTGACA</u> <u>CGTAAAATCTTCGCCGATCTCGGTGCAT</u> <u>GGCAGATTGCGCAACTGGCACGCCATC</u> <u>CACAGCGTCCTTATACCCTGGATTACGT</u> <u>TCGCCTGGCATTGATGAATTTGACGAA</u> <u>CTGGCTGGCGACCGCGGTATGCAGAC</u> <u>GATAAAGCTATCGTC</u></p> | Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of <i>accA</i> gene. |
| DNA fragment used for <i>accBC</i> promoter editing | <p><u>GCTGTTGATTATCTTCCCTGATAAGACCAG</u> <u>TATTTAGCTGCCAATTGCTACGAAATCGTT</u> <u>ATAATGTGCGACCTCGTCCTCCCTGACGC</u> <u>AGTTTTTTCGCTGCGGAAAAGGTGACATT</u> <u>GGCGCAACGAAGGTATATTTTGTTTTTGC</u> <u>CGGAGGATAGCAGCAGATCGCTGCACAAT</u> <u>GTCCGTCAAGTCTAACATTGACACTCTGG</u> <u>GGCAAATAGACCGGCGTCCCGGCCTGCT</u> <u>GGAATTTATCGCTATGCATACAGCTGTGC</u> <u>GGGCATACGCTTTACAGACGGCGGTGAAA</u> <u>CGCCTGTCACATTGACAGCTAGCTCAGTCCT</u> <i>AGGTATAATGCTAGCTAATAGAAATAATTTG</i> <i>TTAACTTTAAAGTTAAGAGGCAAGAATGCAT</i> <u>CATCACCATCACCATGGATATTCGTAA</u> <u>GATTAAAAACTGATCGAGCTGGTTGA</u> <u>AGAATCAGGCATCTCCGAACTGGAAT</u> <u>TTCTGAAGGCGAAGAGTCAGTACGCAT</u> <u>TAGCCGTGCAGCTCCTGCCGCAAGTTT</u> <u>CCCTGTGATGCAACAAGCTTACGCTGC</u></p> | Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of <i>accB</i> gene. |

| | | |
|--|---|--|
| | <p><u>ACCAATGATGCAGCAGCCAGCTCAATC</u> <u>TAACGCAGCCGCTCCGGCGACCGTTCC</u> <u>TTCCATGGAAGCGCCAGCAGCAGCGGA</u> <u>AATCAGTGGTCACATCGTACGTTCCCCG</u> <u>ATGGTTGGTACTTTCTACCGCACCCCAA</u> <u>GCCCGGACGCAAAAGCGTTCATCGAAG</u> <u>TGGGTCAGAAAGTCAACGTGGGCGATA</u> <u>CCCTGTGCATCGTTGAAGCCATGAAAAT</u> <u>GATGAACCAGATCGAAGC</u></p> | |
| <p>DNA fragment used for <i>acs</i> promoter editing</p> | <p><u>CACGACAGTAACCGCACCTACACTGTCAT</u> <u>GACATTGCTCGCCCCTATGTGTAACAAAT</u> <u>AACCACACTGTGAATGTTGTCTTTAATCA</u> <u>ATTGTAAGTGCATGTAATAATACCACTTA</u> <u>GAGTTAGTTAGTATCTTCCTCTTTTTCAAC</u> <u>AGCATGCATAACTGCATGTTCTCAAAGA</u> <u>ATTAATCAACTTTTGTTGCTGACCTTCAA</u> <u>AATTACCCTGCCGTTTATTTGCACAATTCT</u> <u>ACTTTTGCGTGATCTGTCGCCCAAATACTA</u> <u>AACAAAACCTGCCAATACCCCTACATTTAA</u> <u>CGCTTATGCCATTGACAGCTAGCTCAGTCCT</u> <u>AGGTATAATGCTAGCTAATAGAAATAATTTG</u> <u>TTAACTTTAAAGTTAAGAGGCAAGAATGCAT</u> <u>CATCACCATCACCACATGAGCCAAATTCA</u> <u>CAAACACACCATTCTGCCAACATCGCA</u> <u>GACCGTTGCCTGATAAACCCCTCAGCAG</u> <u>TACGAGGCGATGTATCAACAATCTATTA</u> <u>ACGTACCTGATACCTTCTGGGGCGAAC</u> <u>AGGGAAAAATTCTTGACTGGATCAAAC</u> <u>CTTACCAGAAGGTGAAAAACACCTCCTT</u> <u>TGCCCCCGGTAATGTGTCCATTAATGG</u> <u>TACGAGGACGGCACGCTGAATCTGGCG</u> <u>GCAAACCTGCCTTGACCGCCATCTGCAA</u> <u>GAAAACGGCGATCGTACCGCCATCATC</u> <u>TGGGAAGGCGACGACGCCAGCCAGAGC</u> <u>AAACATATCAGC</u></p> | <p>Underlined letters are upstream and downstream homologous arms, italic letters are J23119 promoter region, bold letters are part of the coding region of <i>acs</i> gene.</p> |

Information of genes used in this study:

Gene: *At4CL*

GenBank: KX817185.1

Codon optimized DNA sequence:

ATGGCGCCGCAGGAACAGGCGGTTTCTCAGGTTATGGAAAAACAGTCTAA
CAACAACAACCTCTGACGTTATCTTCCGTTCTAAACTGCCGGACATCTACAT
CCCGAACCACCTGTCTCTGCACGACTACATCTTCCAGAACATCTCTGAATT
CGCGACCAAACCGTGCCTGATCAACGGTCCGACCGGTCACGTTTACACCT
ACTCTGACGTTACGTTATCTCTCGTCAGATCGCGGCGAACTTCCACAAAC
TGGGTGTTAACCAGAACGACGTTGTTATGCTGCTGCTGCCGAACTGCCCG
GAATTCGTTCTGTCTTTCCTGGCGGCGTCTTTCGTTGGTGCACCGCGACC
GCGGCGAACCCTTCTTACCCCGGCGGAAATCGCGAAACAGGCGAAAG
CGTCTAACACCAAACCTGATCATCACCGAAGCGCGTTACGTTGACAAAATC
AAACCGCTGCAGAACGACGACGGTGTGTTATCGTTTGCATCGACGACAA
CGAATCTGTTCCGATCCCGGAAGGTTGCCTGCGTTTACCGAACTGACCCA
GTCTACCACCGAAGCGTCTGAAGTTATCGACTCTGTTGAAATCTCTCCGGA
CGACGTTGTTGCGCTGCCGTACTCTTCTGGTACCACCGGTCTGCCGAAAGG
TGTTATGCTGACCCACAAAGGTCTGGTTACCTCTGTTGCGCAGCAGGTTGA
CGGTGAAAACCCGAACCTGTACTTCCACTCTGACGACGTTATCCTGTGCGT
TCTGCCGATGTTCCACATCTACGCGCTGAACTCTATCATGCTGTGCGGTCT
GCGTGTGTTGGTGCAGGCGATCCTGATCATGCCGAAATTCGAAATCAACCTGC
TGCTGGAACCTGATCCAGCGTTGCAAAGTTACCGTTGCGCCGATGGTTCCG
CCGATCGTTCTGGCGATCGCGAAATCTTCTGAAACCGAAAAATACGACCT
GTCTTCTATCCGTGTTGTTAAATCTGGTGCAGGCGCCGCTGGGTAAAGAACT
GGAAGACGCGGTTAACGCGAAATTCGGAACGCGAAACTGGGTGAGGGT
TACGGTATGACCGAAGCGGGTCCGGTCTGGCGATGTCTCTGGGTTTCGC
GAAAGAACCCTTCCCGTTAAATCTGGTGCCTGCGGTACCGTTGTTTCGTA
ACGCGGAAATGAAAATCGTTGACCCGGACACCGGTGACTCTCTGTCTCGT
AACCAGCCGGGTGAAATCTGCATCCGTGGTACCAGATCATGAAAGGTTA
CCTGAACAACCCGGCGGCGACCCGCGGAAACCATCGACAAAGACGGTTGG
CTGCACACCGGTGACATCGGTCTGATCGACGACGACGACGAACTGTTTCAT
CGTTGACCGTCTGAAAGAACTGATCAAATACAAAGGTTTCCAGGTTGCGC
CGGCGGAACTGGAAGCGCTGCTGATCGGTCACCCGGACATCACCGACGTT
GCGGTTGTTGCGATGAAAGAAGAAGCGGCGGGTGAAGTTCCGGTTGCGTT
CGTTGTTAAATCTAAAGACTCTGAACTGTCTGAAGACGACGTTAAACAGT
TCGTTTCTAAACAGGTTGTTTCTACAAACGTATCAACAAAGTTTTCTTCA
CCGAATCTATCCCGAAAGCGCCGTCTGGTAAAATCCTGCGTAAAGACCTG
CGTGCGAAACTGGCGAACGGTCTGTAA

Gene: *VvSTS*

GenBank: NM_001281010.1

Codon optimized DNA sequence:

ATGGCGTCTGTTGAAGAATTCCGTAACGCGCAGCGTGCGAAAGGTCCGGC
GACCATCCTGGCGATCGGTACCGCGACCCCGGACCACTGCGTTTACCAGT
CTGACTACGCGGACTACTACTTCCGTGTTACCAAATCTGAACACATGACC
GAACTGAAAAAAAATTCAACCGTATCTGCGACAAATCTATGATCAAAAA
ACGTTACATCCACCTGACCGAAGAAATGCTGGAAGAACCCCGAACATCG
GTGCGTACATGGCGCCGTCTCTGAACATCCGTCAGGAAATCATCACCGCG
GAAGTTCGCGCTCTGGGTCGTGACGCGGCGCTGAAAGCGCTGAAAGAATG
GGGTCAGCCGAAATCTAAAATCACCCACCTGGTTTTCTGCACCACCTCTGG
TGTTGAAATGCCGGGTGCGGACTACAACTGGCGAACCTGCTGGGTCTGG
AAACCTCTGTTTCGTCTGTTATGCTGTACCACCAGGGTTGCTACGCGGGTG
GTACCGTTCGCGTACCGCGAAAGACCTGGCGGAAAACAACGCGGGGTGCG
CGTGTTCTGGTTGTTTGCTCTGAAATCACCGTTGTTACCTCCGTGGTCCGT
CTGAAGACGCGCTGGACTCTCTGGTTGGTCAGGCGCTGTTTCGGTGACGGT
TCTTCTGCGGTTATCGTTGGTTCTGACCCGGACGTTTCTATCGAACGTCCG
CTGTTCCAGCTGGTTTCTGCGGCGCAGACCTTCATCCCGAACTCTGCGGGT
GCGATCGCGGGTAACCTGCGTGAAGTTGGTCTGACCTTCCACCTGTGGCC
GAACGTTCCGACCCTGATCTCTGAAAACATCGAAAAATGCCTGACCCAGG
CGTTCGACCCGCTGGGTATCTCTGACTGGAACCTCTCTGTTCTGGATCGCGC
ACCCGGGTGGTCCGGCGATCCTGGACGCGGTTGAAGCGAACTGAACCTG
GAAAAAAAATACTGGAAGCGACCCGTCACGTTCTGTCTGAATACGGTAA
CATGTCTTCTGCGTGCGTTCTGTTTCATCCTGGACGAAATGCGTAAAAAATC
TCTGAAAGGTGAAAAAGCGACCACCGGTGAAGGTCTGGACTGGGGTGTTT
TGTTTCGGTTTCGGTCCGGGTCTGACCATCGAAACCGTTGTTCTGCACTCTG
TTCCGACCGTTACCAACTAA

Gene: *IacE*

GenBank: XM_007832623.1

DNA sequence:

ATGGCTATCTCAACCCCTTCCAACGGCGTAAGCCATGTCGCAAAGCCTCT
ACCCAACCTGAAAGAGGTCAATAAAGGCATTGAGACAGACAGTGAAGAC
CGAGCCTTCTGGTGGGGTGCCTTGTCTGAGCCTTTGGCCTCGCTCCTCGAG
GCAAATCACTACACCAAGGAAGTGCAACTTCACTACCTCCGTTGGTTTTAC
CAATGGATTCTACCGGCTCTGGGGCCACGACCTCTCGACGGCAAACCCTA
TTACGGGTCTTGGATTACTCATGATTTGTCGCCCTTTGAGTACAGTCTCAA
TTGGAAAGAAAAGAGTTCCAAACAGACCATCCGTTTCACTATTGAGGCGG

TTACGAAGCAGTCTGGCACAGCCAGCGATCCTATCAACCAGCTCGGGCGCA
AAGGAGTTCCTGGAAGCAGTCTCCAAGGATGTTCTTGGTATGGATCTCAC
GCGATTCAACCAGTTTCTTGAGGCAACCAACGTACCCAACGACTGCGTAG
ATGATGCCATTGCAAAGCACCCAGCACACTTCCCTCGCAGCCGCGTTTGG
ATCGCTTTTGACTTGGAGCACTCTGGCAACCTTATGGCCAAGTCATACTTC
CTGCCGCATTGGCGTGCGATCCAGAGCGGTATCTCGGCCAATACCATCAT
TGCGGATACGGTCAAGGAGTGCAACAAGGCGGACGGTTCGTCTGACGAC
GGGTGCTGAACGCCATTGAGTCGTACCTGGCAACGTTACGCGACCGGA
GGAGGCGCCGCAGATGGGACTGCTGTCAAATGACTGCGTGGCCGAAACG
CCCGGCTCGCGCCTCAAGGTCTATTTCCGCTCCTCGGCGGACACGCTCGCC
AAGGCCAAGGACATGTACAACCTGGGTGGCCGCCTGAAGGGCCCAAGA
TGGATGCCAGCCTCAAGGGTATCAGTGATTTCTGGTACCACCTGTTTGGCC
TCGACAGCTCCGACCCGGCCTCTGATGACAAGGTCTGTATCGGCAACCAC
AAGTGCATTTTCGTCTACGAGATGCGCTCCTCGCAAGGCTCGGAGCCGA
TATTGATGTCAAGTTCCACATCCCAATGTGGCAGCTTGGTAAGACGGATG
GGCAGATTAGCGAGCTGCTGGCTTCTTGGTTCGAGAGCCATGGTCACCCT
GATCTCGCCTCGAGGTATAAGTCTGACTTGGGCACGGCATTTCCTCAAGCA
CAATATTACGGGCAAGAGTGTAGGAACCCATACTTACATCTCCATCACTC
ACACACCCAAGACGGGATTGTACATGACCATGTATCTCAGCCCGAAACTG
CCCGAGTTCTACTACTAA

Gene: *accA*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

ATGAGTCTGAATTCCTTGATTTTGAACAGCCGATTGCAGAGCTGGAAGC
GAAAATCGATTCTCTGACTGCGGTTAGCCGTCAGGATGAGAACTGGATA
TTAACATCGATGAAGAAGTGCATCGTCTGCGTGAAAAAAGCGTAGAACTG
ACACGTAAAATCTTCGCCGATCTCGGTGCATGGCAGATTGCGCAACTGGC
ACGCCATCCACAGCGTCCTTATACCCTGGATTACGTTTCGCTGGCATTGA
TGAATTTGACGAACTGGCTGGCGACCGCGCGTATGCAGACGATAAAGCTA
TCGTTCGGTGGTATCGCCCGTCTCGATGGTTCGTCCGGTGGTATGATCATTGGTC
ATCAAAAAGGTCGTGAAACCAAAGAAAAAATTCGCCGTAACTTTGGTATG
CCAGCGCCAGAAGGTTACCGCAAAGCACTGCGTCTGATGCAAATGGCTGA
ACGCTTTAAGATGCCAATCATCACCTTTATCGACACCCCGGGGGCTTATCC
GGGCGTGGGCGCAGAAGAGCGCGGTTCAGTCTGAAGCCATTGCACGCAAC
CTGCGTGAAATGTCTCGCCTTAGCGTACCGACTATTTGTACCGTTATCGGT
GAAGGTGGTTCTGGCGGCGCGCTGGCGATTGGCGTGGGCGATAAAGTGAA
TATGCTGCAATACAGCACCTATTCCGTTATCTCGCCGGAAGGTTGTGCGTC
CATTCTGTGGAAGAGCGCTGATAAAGCGCCGCTGGCGGCTGAAGCGATGG
GTATCATCGCTCCGCGTCTGAAAGAATAAACTGATCGACTCCATCATC

CCAGAACCGCTGGGTGGTGCTACCGTAACCCGGAAGCGATGGCGGCATC
GTTGAAAGCGCAACTGTTGGCGGATCTGGCCGATCTCGACGTGTTAAGCA
CTGAAGATTTAAAAAATCGTCGTTATCAGCGCCTGATGAGCTACGGTTAC
GCGTAA

Gene: *accBC*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence (the *AccB* coding sequence was underlined, *AccC* coding sequence was in bold):

ATGGATATTCGTAAGATTA**AAAAA**ACTGATCGAGCTGGTTGAAGAATCAGG
CATCTCCGAACTGGAAATTTCTGAAGGCGAAGAGTCAGTACGCATTAGCC
GTGCAGCTCCTGCCGCAAGTTTCCCTGTGATGCAACAAGCTTACGCTGCAC
CAATGATGCAGCAGCCAGCTCAATCTAACGCAGCCGCTCCGGCGACCGTT
CCTTCCATGGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCACATCGTACG
TTCCCCGATGGTTGGTACTTTCTACCGCACCCCAAGCCCGGACGCAAAAAG
CGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACCCTGTGCATC
GTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTA
CCGTGAAAGCAATTCTGGTTCGAAAGTGGACAACCGGTAGAATTTGACGAG
CCGCTGGTCGTCATCGAGTAA**CGAGGCGAACATGCTGGATAAAAATTGTT**
ATTGCCAACCGCGGGCGAGATTGCATTGCGTATTCTTCGTGCCTGTAAA
GAACTGGGCATCAAGACTGTCGCTGTGCACTCCAGCGCGGATCGCGA
TCTAAAACACGTATTACTGGCAGATGAAACGGTCTGTATTGGCCCTGC
TCCGTCAGTAAAAAGTTATCTGAACATCCCGGCAATCATCAGCGCCG
CTGAAATCACCGGCGCAGTAGCAATCCATCCGGGTTACGGCTTCCTC
TCCGAGAACGCCAACTTTGCCGAGCAGGTTGAACGCTCCGGCTTTAT
CTTCATTGGCCC**GAAAGCAGAAACCATT****CGCCTGATGGGCGACAAAG**
TATCCGCAATCGCGGCGATGAAAAAAGCGGGCGTCCCTTGCGTACCG
GGTTCGACGGCCCGCTGGGCGACGATATGGATAAAAACCGTGCCAT
TGCTAAACGCATTGGTTATCCGGTGATTATCAAAGCCTCCGGCGGGCG
GCGGCGGTTCGCGGTATGCGCGTAGTGCGCGGGCGACGCTGAACTGGC
ACAATCCATCTCCATGACCCGTGCGGAAGCGAAAGCTGCTTTCAGCA
ACGATATGGTTTACATGGAGAAATACCTGGAAAATCCTCGCCACGTC
GAGATTCAGGTA**CTGGCTGACGGTCAGGGCAACGCTATCTATCTGGC**
GGAACGTGACTGCTCCATGCAACGCCGCCACCAGAAAGTGGT**CGAAG**
AAGCGCCAGCACCGGGCATTACCCCGGAACTGCGT**CGCTACATCGGC**
GAACGTTGCGCTAAAGCGTGTGTTGATATCGGCTATCGCGGTGCAGG
TACTTTCGAGTTCCTGTT**CGAAAACGGCGAGTTCTATTT****CATCGAAAT**
GAACACCCGTATTCAGGTAGAACACCCGGTTACAGAAATGATCACCG
GCGTTGACCTGATCAAAGAACAGCTGCGTATCGCTGCCGGTCAACCG

CTGTTCGATCAAGCAAGAAGAAGTTCACGTTTCGCGGCCATGCGGTGGA
ATGTTCGTATCAACGCCGAAGATCCGAACACCTTCCTGCCAAGTCCGG
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GAGTCTCATATCTACGCGGGCTACACCGTACCGCCGTAATGACTC
AATGATCGGTAAGCTGATTTGCTACGGTGAAAACCGTGACGTGGCGA
TTGCCCGCATGAAGAATGCGCTGCAGGAGCTGATCATCGACGGTATC
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CCAGCATGGTGGCACTAACATCCACTATCTGGAGAAAAAACTCGGTC
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Gene: *acs*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

ATGAGCCAAATTCACAAACACACCATTTCCTGCCAACATCGCAGACCGTTG
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CCTTACCAGAAGGTGAAAAACACCTCCTTTGCCCCCGTAATGTGTCCATT
AAATGGTACGAGGACGGCACGCTGAATCTGGCGGCAAACCTGCCTTGACCG
CCATCTGCAAGAAAACGGCGATCGTACCGCCATCATCTGGGAAGGCGACG
ACGCCAGCCAGAGCAAACATATCAGCTATAAAGAGCTGCACCGCGACGTC
TGCCGCTTCGCCAATACCCTGCTCGAGCTGGGCATTAAAAAAGGTGATGT
GGTGGCGATTTATATGCCGATGGTGCCGGAAGCCGCGGTTGCGATGCTGG
CCTGCGCCCGCATTGGCGCGGTGCATTCGGTGATTTTCGGCGGCTTCTCGC
CGGAAGCCGTTGCCGGGCGCATTATTGATTCCAACCTCACGACTGGTGATC
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CGTTGATGACGCGCTGAAAAACCCGAACGTCACCAGCGTAGAGCATGTGG
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TCCCTGCCTAAAACCCGCTCCGGCAAATTATGCGCCGTATTCTGCGCAA
AATTGCGGGCGGGCGATAACCAGCAACCTGGGCGATACCTCGACGCTTGCCG
ATCCTGGCGTAGTCGAGAAGCTGCTTGAAGAGAAGCAGGCTATCGCGATG
CCATCGTAA

Gene: *gltA*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

ATGGCTGATACAAAAGCAAACTCACCCCTCAACGGGGACACAGCTGTTGA
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CTCTCGGTTCAAAGGTGTGTTACCTTTGACCCTGGCTTCACTTCAACCG
CATCCTGCGAATCTAAAATTACTTTTATTGATGGTGATGAAGGTATTTTGC
TGCACCGCGGTTTCCCGATCGATCAGCTGGCGACCGATTCTAACTACCTGG
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GTACCGTTGGCTGGATCGCCCACTGGAGCGAGATGCACAGTGACGGTATG
AAGATTGCCCGTCCGCGTCAGCTGTATACAGGATATGAAAAACGCGACTT
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Gene: *acpS*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

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GCGAAGCGTTTTGCTGTGAAAGAAGCCGCAGCAAAGCGTTTGGCACCGG
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CGGCAAACCACGGCTACGGCTATGGGGCGAGGCATTAAACTGGCGGAA
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TGCTTGTGCCACGGTAATTATTGAAAGTTAA

Gene: *acpT*

GenBank: NC_012892 (*E. coli* BL21(DE3) genome)

DNA sequence:

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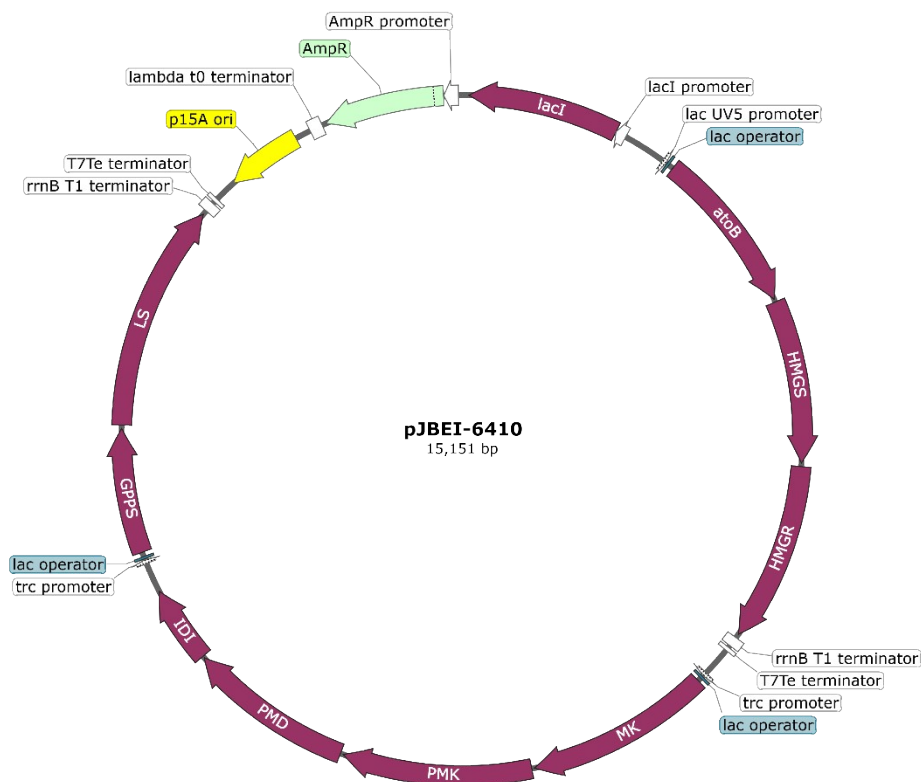


Fig. S1 The structure illustration of plasmid pJBEI-6410. The MTSA operon containing genes for the conversion of acetyl CoA to mevalonate was expressed by lac UV5 promoter. AtoB: acetoacetyl-CoA synthase from *E. coli*; HMGS: HMG-CoA synthase from *Staphylococcus aureus* codon optimized for *E. coli*; HMGR: HMG-CoA reductase from *S. aureus* codon optimized for *E. coli*. MBI operon containing genes for the conversion of mevalonate to isoprenoid precursors (IPP and DMAPP) was expressed by trc promoter. MK: Mevalonate Kinase, PMK: Phosphomevalonate Kinase, and PMD: Phosphomevalonate Decarboxylase were from *Saccharomyces cerevisiae*; IDI: isopentenyl diphosphate isomerase from *E. coli*. GPPS: Geranyl pyrophosphate synthase from *Abies grandis* and codon optimized for *E. coli*; LS: Limonene synthase from *Mentha spicata*, a truncated version without plastidial targeting sequence and codon optimized for *E. coli*²⁹.

Plasmid sequence of pJBEI-6410:

gacgtcggcctaataagtagtaacttacattaattgcgttgcgctcactgccgcttccagtcgggaaacctgctg
ccagctgcattaatgaatcgccaacgcgcggggagagggcgtttgcgtattggggccaggggtggttttctttaccag
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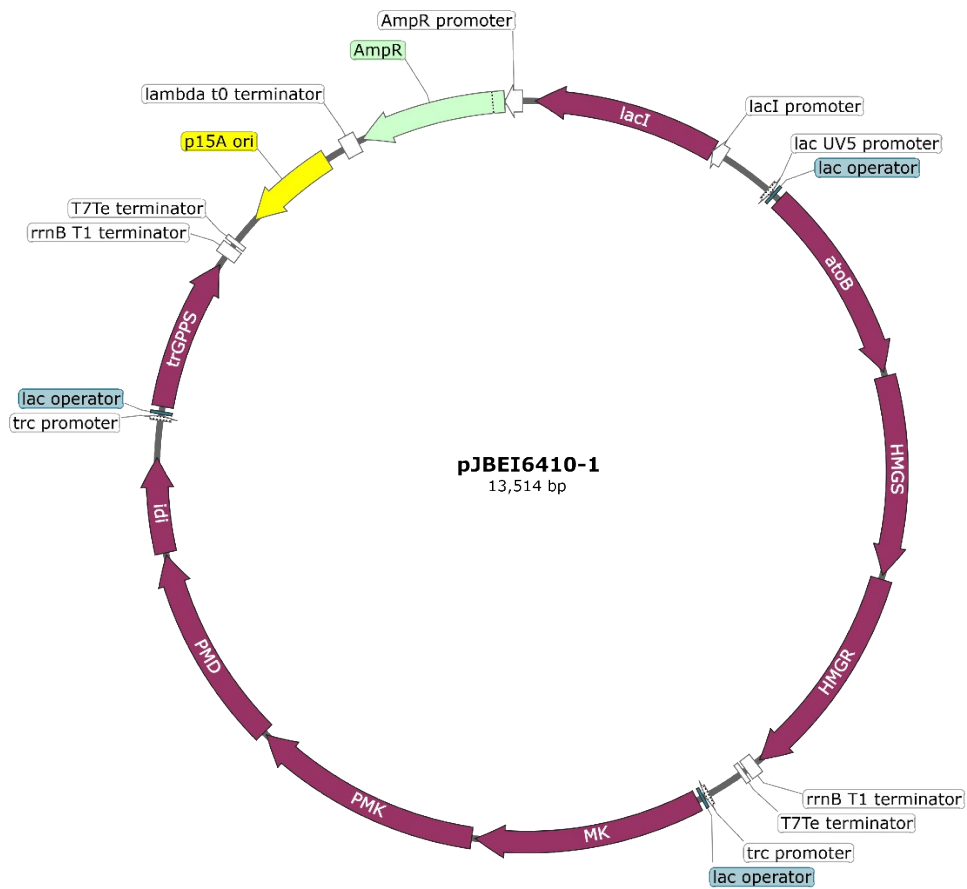


Fig. S2 The structure illustration of plasmid pJBEI6410-1. In this plasmid, limonene synthase gene was deleted for GPP supplementation.

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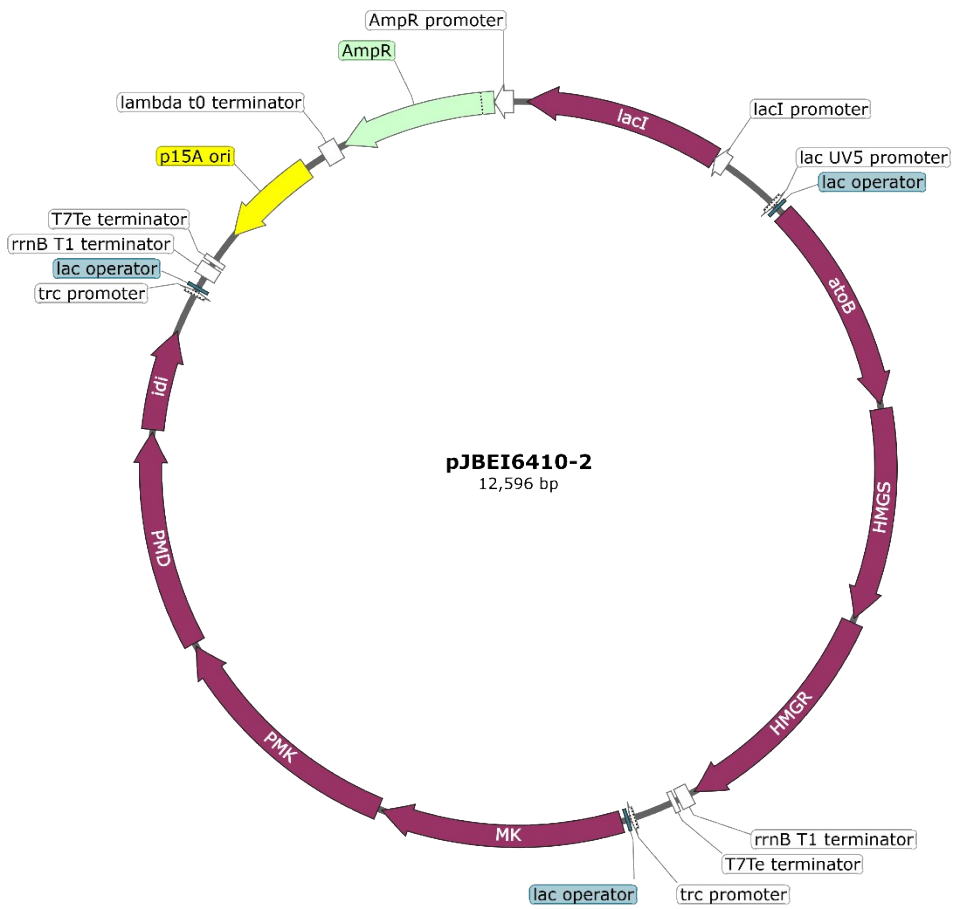


Fig. S3 The structure illustration of plasmid pJBEI6410-2. In this plasmid, the geranyl pyrophosphate synthase gene was further deleted for DMAPP supplementation.

Plasmid sequence of pJBEI6410-2:

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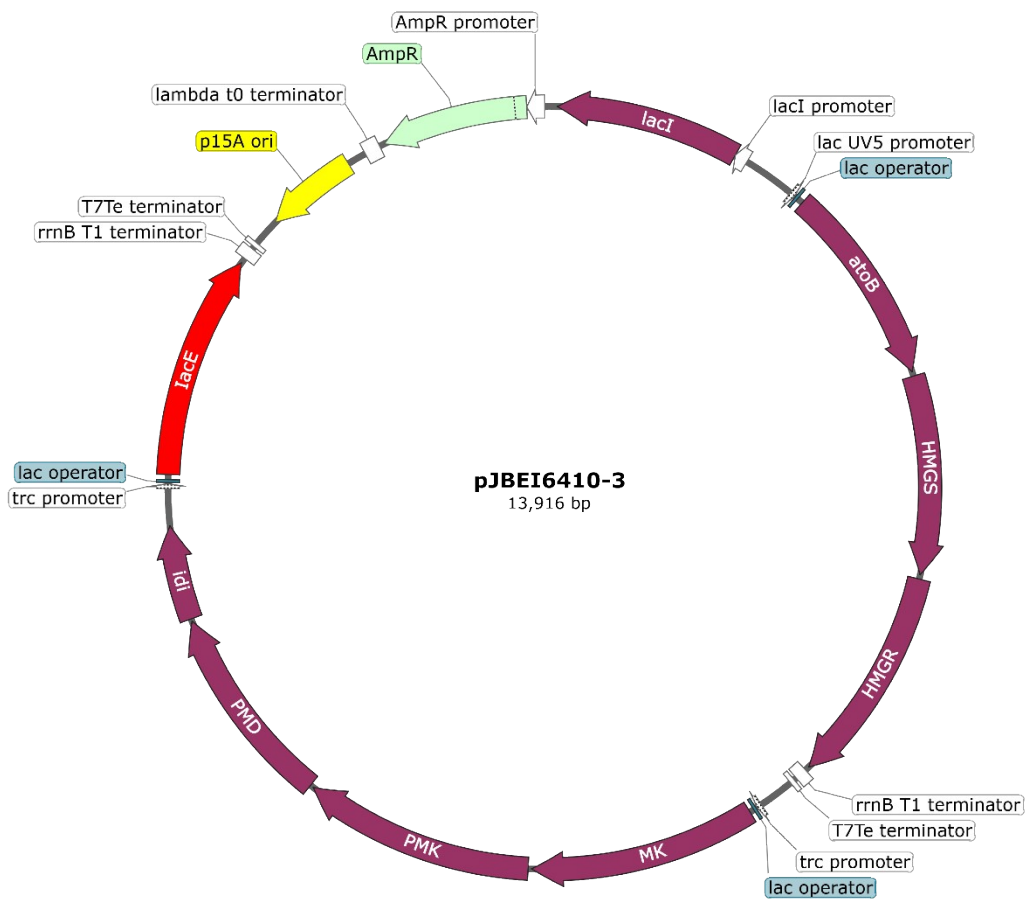


Fig. S4 The structure illustration of plasmid pJBEI6410-4. In this plasmid, the prenyltransferase *IacE* was inserted after the *trc* promoter.

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Fig. S5 The structure illustration of plasmid pCDFRESa. The codon optimized At4CL was inserted between restriction sites BamHI and NotI, so that it was expressed with a 6 × His tag at the N-terminal. The codon optimized VvSTS was inserted between restriction sites NdeI and XhoI.

Plasmid sequence of pCDFRESa:

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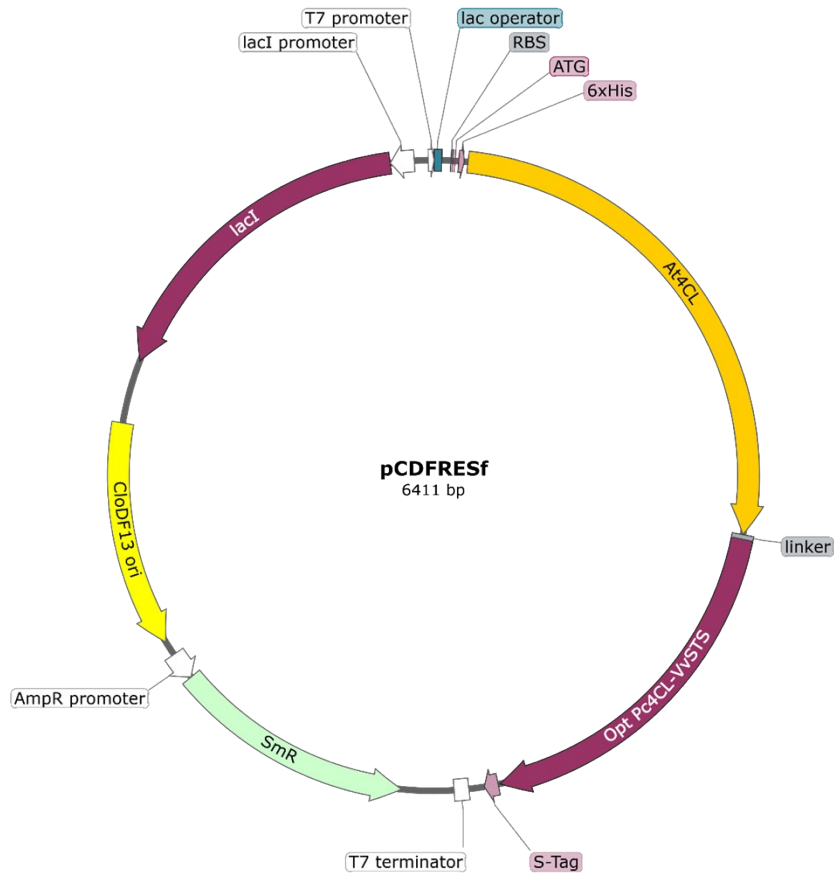


Fig. S6 The structure illustration of plasmid pCDFRESf. The codon optimized At4CL and VvSTS were expressed as a fusion protein, linked with a rigid linker (EAAAK) between the C-terminal of At4CL and the N-terminal of VvSTS. The fusion protein was inserted between restriction sites BamHI and NotI, so that it was expressed with a 6 × His tag at the N-terminal.

cgctcgatgacccaactacctgatagttgagtcgatactcggcgatcaccgctccctcatactctccttttcaatattattgaagcattat
cagggttattgtctcatgagcggatacatattgaaatgatttagaaaaataacaaatagctagctcactcggctcgtacgctccggggtgag
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attaggaattaatacgaactactata



Fig. S7 The structure illustration of plasmid pETlac-asgltA. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

Plasmid sequence of pETlac-asgltA:

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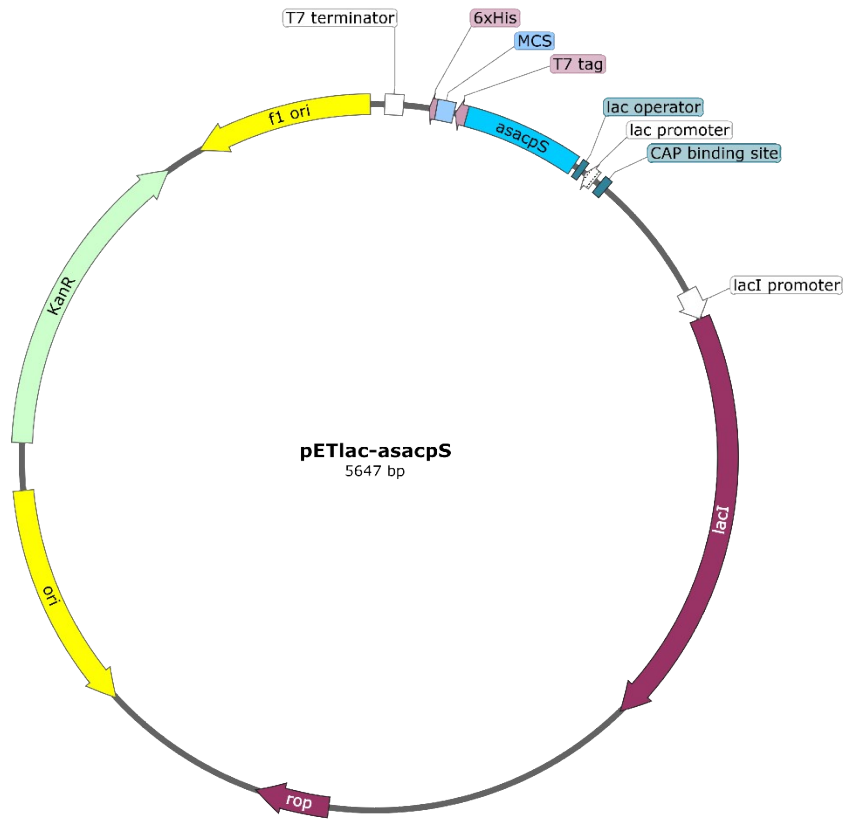


Fig. S8 The structure illustration of plasmid pETlac-asacpS. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

Plasmid sequence of pETlac-asacpS:

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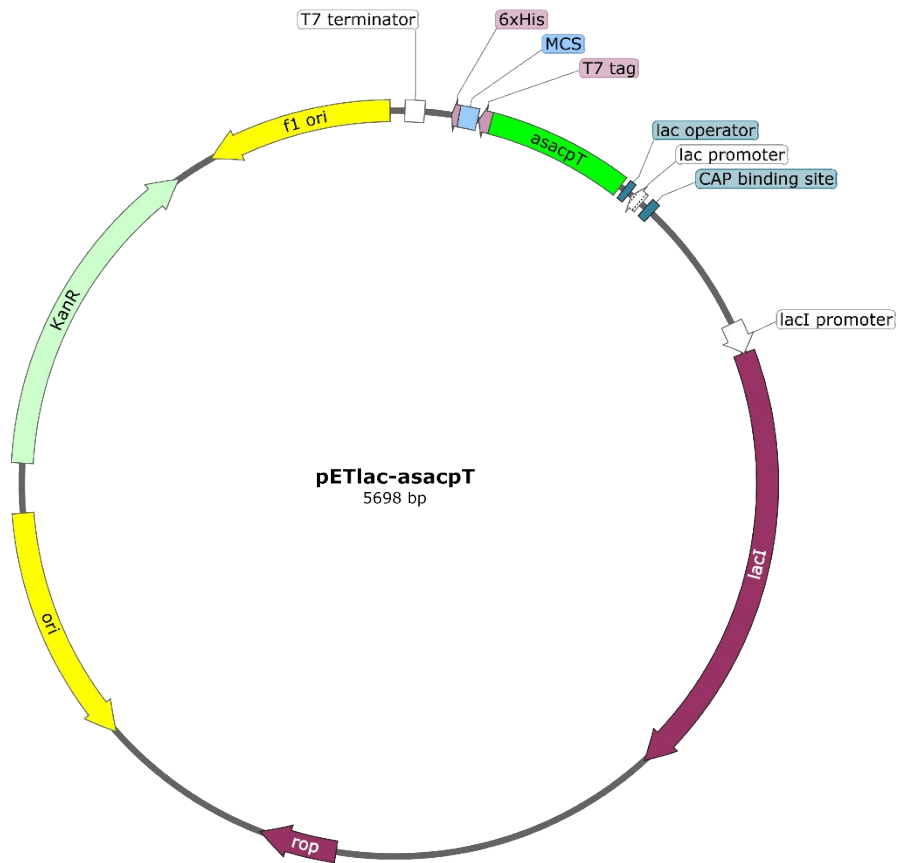


Fig. S9 The structure illustration of plasmid pETlac-asacpT. The T7 promoter was replaced by lac promoter amplified from pUC19, followed with the anti-gltA RNA frame.

Plasmid sequence of pETlac-asacpT:

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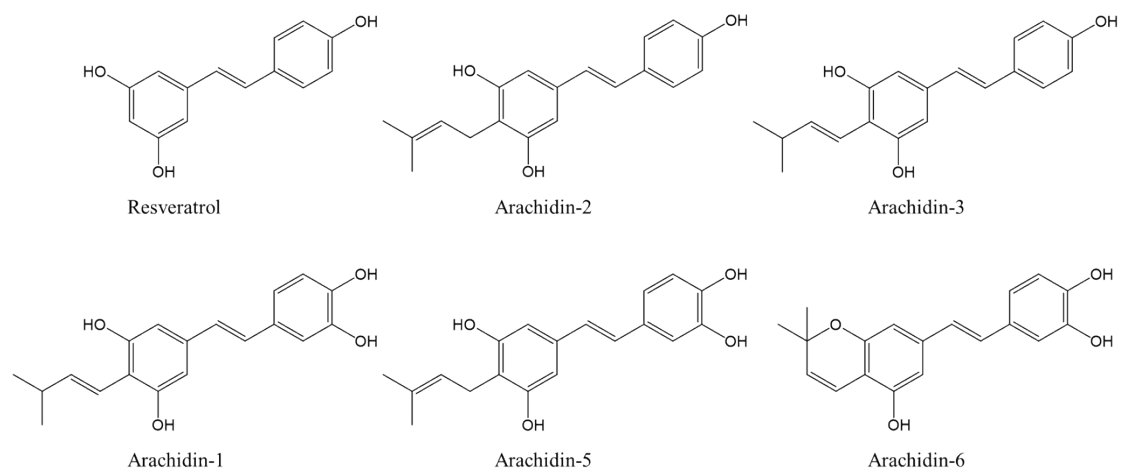


Fig. S10 Resveratrol and the prenylated stilbenoids isolated from the elicited hairy root cultures and seedlings of peanut³⁸⁻⁴⁰.

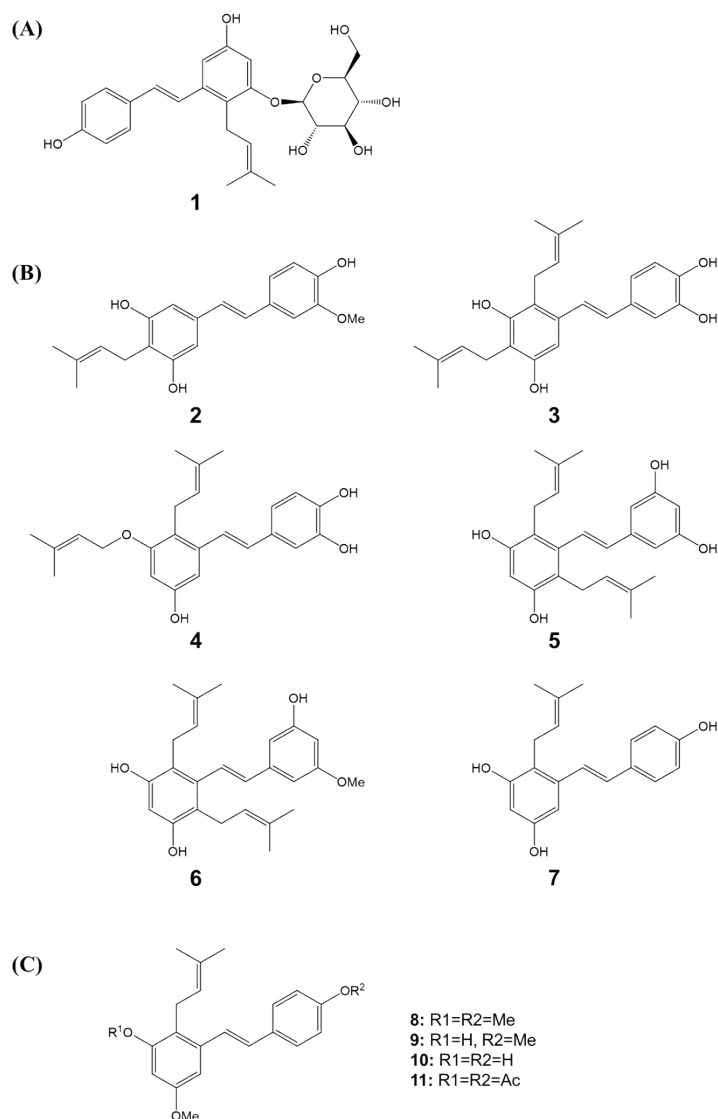


Fig. S11 2-C-prenyl resveratrol and its derivatives. (A) Vulpinoideol A (**1**) from *Carex vulpinoidea*⁴². (B) (*E*)-4-(3-methyl-2-buten-1-yl)-3,4',5-trihydroxy-3'-methoxystilbene (**2**), (*E*)-2,4-bis(3-methyl-2-buten-1-yl)-3,3',4',5-tetrahydroxystilbene (**3**), (*E*)-2-(3-methyl-2-buten-1-yl)-3-(3-methyl-2-butenyloxy)-3',4',5-trihydroxystilbene (**4**), (*E*)-2,6-bis(3-methyl-2-buten-1-yl)-3,3',5,5'-tetrahydroxystilbene (**5**) and (*E*)-2,6-bis(3-methyl-2-buten-1-yl)-3,4',5-trihydroxy-3'-methoxystilbene (**6**), and 2-C-prenyl resveratrol (**7**) isolated from Kangaroo Island propolis⁴¹. (C) 2-prenyl-3,5,4'-trimethoxystilbene (**8**), 2-prenyl-3-hydroxy-5,4'-dimethoxystilbene (**9**) and 2-prenyl-3,4'-dihydroxy-5-methoxystilbene (**10**) and its corresponding acetate (**11**) isolated from *Scirpus holoschoenus* L.⁴³.

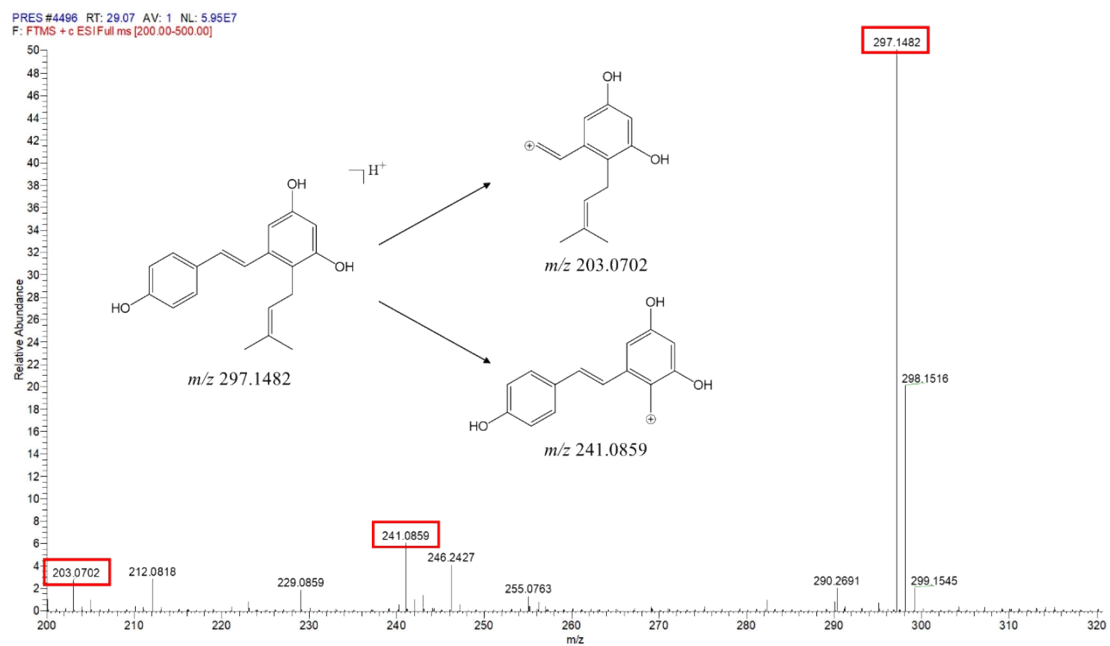


Figure S12. The ESI-MS spectrum and putative fragmentation pathway of the product 2-C-prenyl resveratrol.

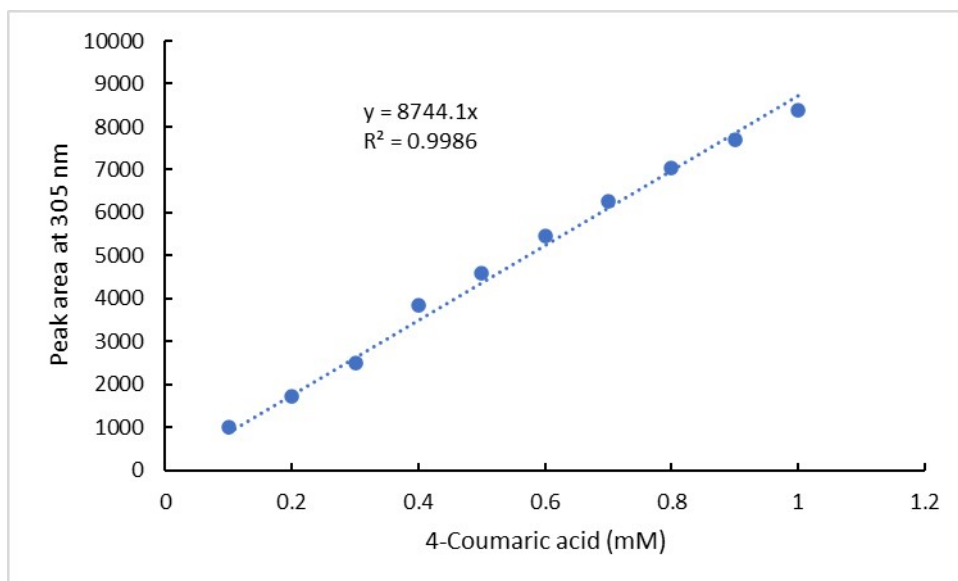


Fig. S13 Standard curve for 4-coumaric acid.

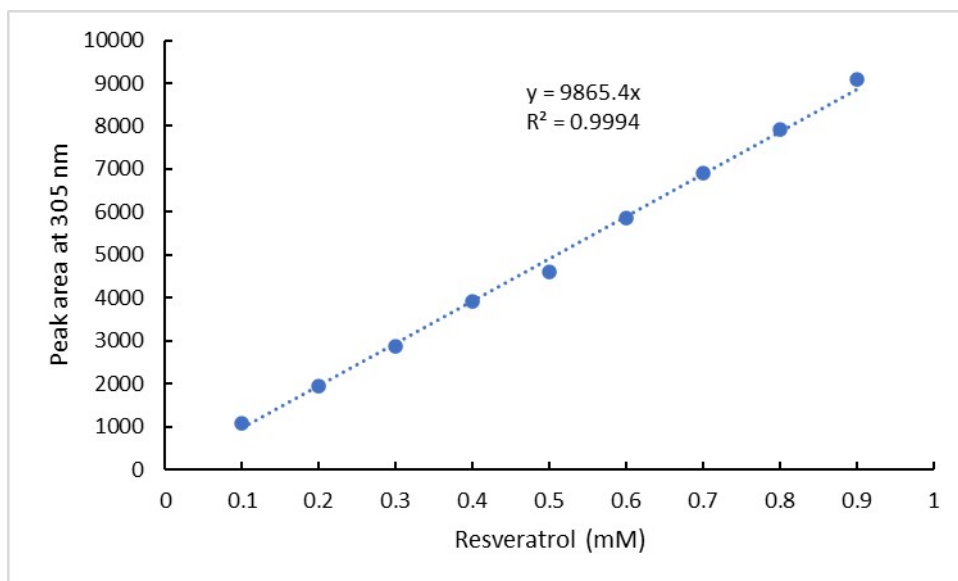


Fig. S14 Standard curve for resveratrol.

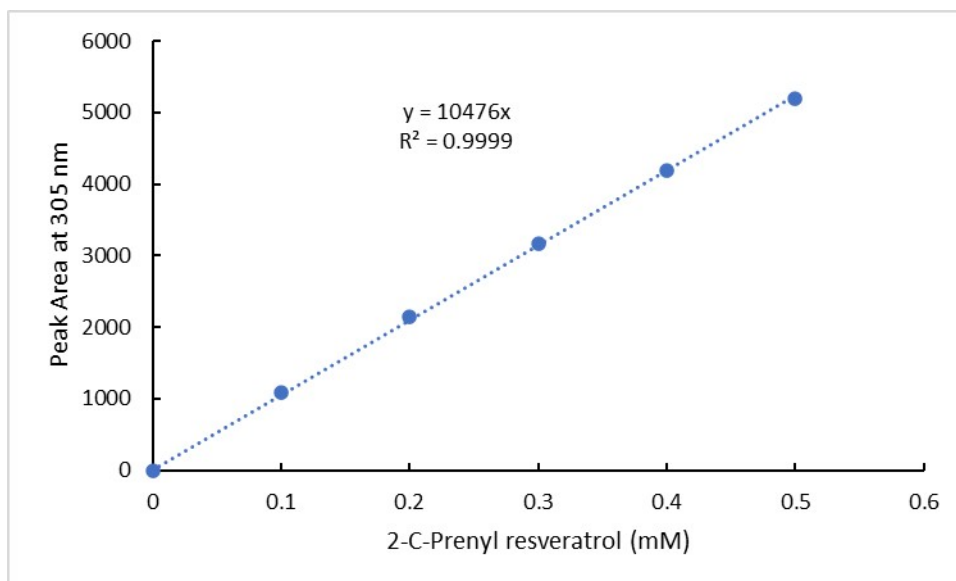


Fig. S15 Standard curve for 2-C-prenyl resveratrol.

Media compositions:

LB: tryptone 10 g/L, sodium chloride 10 g/L, yeast extract 5 g/L, sterilized at 121 °C for 20 min.

Minimal M9: disodium hydrogen phosphate 6.8 g/L, potassium dihydrogen phosphate 3.0 g/L, sodium chloride 0.5 g/L, ammonium chloride 1.0 g/L, calcium chloride 0.01 g/L, sterilized at 121 °C for 20 min. The medium was supplemented with glucose 10 g/L, magnesium sulfate 0.12 g/L before use. Glucose and magnesium sulfate were sterilized separately at 115 °C for 30 min.

TB: tryptone 12 g/L, yeast extract 24 g/L, glycerol 4 mL/L, dipotassium phosphate 12.54 g/L, potassium dihydrogen phosphate 2.31 g/L, sterilized at 121 °C for 20 min.

EZ Rich Defined Medium was purchased from Coolaber (Beijing) Technology (product No. MK0106) containing 10×MOPS mixture, 10×ACGU, 5×Supplement EZ, diluted with sterilized water and supplemented with 0.23 g/L sterilized dipotassium phosphate and 2 g/L glucose.

Mineral medium: diammonium hydrogen phosphate 4 g/L, potassium dihydrogen phosphate 13.54 g/L, citric acid 1.7 g/L, yeast extract 5 g/L, sterilized at 121 °C for 20 min. The medium was supplemented with glucose 10g/L, magnesium sulfate 0.7 g/L, 200×trace elements 5ml/L before use. Glucose and magnesium sulfate were sterilized separately at 115 °C for 30 min. 200×trace elements were sterilized through filtration with 0.22 µm filter.

200×trace elements: ferrous sulfate 11 g/L, calcium chloride 4 g/L, copper sulfate 1.28 g/L, zinc sulfate 2.47 g/L, ammonium heptamolybdate 0.19 g/L, manganese sulfate 0.68 g/L, sodium tetraborate 0.02 g/L, dissolved in 1 M HCl filtered with 0.22 µm filter.