

Electronic Supplementary Information

Constructing a green oleaginous yeast cell factory for sustainable production of the plant-derived diterpenoid sclareol

Mei-Li Sun^a, Yuting Han^a, Xiao Yu^a, Kaifeng Wang^a, Lu Lin^a, Rodrigo Ledesma-Amaro^b, Xiao-Jun Ji^{a*}

^a State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, No. 30 South Puzhu Road, Nanjing 211816, People's Republic of China

^b Department of Bioengineering and Imperial College Centre for Synthetic Biology, Imperial College London, London SW7 2AZ, UK

Correspondence: xiaojunji@njtech.edu.cn (X.-J. Ji)

Supplementary Methods:

Analytical methods of dodecane proportion in fed-batch fermentation

In fed-batch fermentation, approximately 20% dodecane was added for the extraction of sclareol, resulting in a 5-fold product concentration in the dodecane phase. However, the dodecane proportion changed with periodic sampling and subsequent feeding, which had a direct effect on sclareol quantification. To attain the accurate titer of sclareol generated by the strain to the maximum extent possible, the dodecane ratio was corrected as follows:

A) Before sampling, the total volume of culture was V_{cn} ml, the volume of dodecane was V_{dn-1} ml, so the dodecane ratio a_n was:

$$a_n = \frac{V_{dn}}{V_{cn}}$$

B) Then, a sample of V_{rn+1} ml was taken from the bioreactor. Assuming a homogeneous system under high-speed stirring, the dodecane ratio of the sample was a_n . If the glucose, NaOH solution and antifoam was fed V_{fn+1} after sampling, the dodecane ratio a_{n+1} was:

$$a_{n+1} = \frac{V_{dn+1}}{V_{cn+1}} = \frac{V_{dn} - V_{rn+1} \times a_n}{V_{cn} + V_{fn+1} - V_{rn+1} \times (1 - a_n)}$$

C) If the dodecane phase was diluted b_n times for gas chromatogram, the sclareol titer was measured as c_{mn} mg/L, and the accurate titer of sclareol (c_n mg/L) in this sampling was:

$$c_n = a_n \times b_n \times c_{mn}$$

Supplementary Table 1. Plasmids used in this study.

Plasmids	Genotype or properties	Sources
pUC-Leu-A08-CcCLS	P _{TEFin} -CcCLS-T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-Leu-A08-SsLPPS	P _{TEFin} -SsLPPS-T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-HUH-IntE2-SsSCS	P _{TEFin} -SsSCS-T _{xpr2t} cassette in pUC-HUH-IntE2	This study
pUC-HUH-IntC-tHMG	P _{TEFin} -tHMG-T _{xpr2t} cassette in pUC-HUH-IntC	1
pUC-HUH-SCP2-ERG20	P _{TEFin} -ERG20-T _{xpr2t} cassette in pUC-HUH-SCP2	1
pUC-Leu-A08-dCcCLS	P _{TEFin} -dCcCLS-T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-Leu-A08-dSsLPPS	P _{TEFin} -dSsLPPS-T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-HUH-IntE2-dSsSCS	P _{TEFin} -dSsSCS-T _{xpr2t} cassette in pUC-HUH-IntE2	This study
pUC-HUH-IntA-YIGGPPS	P _{TEFin} -YIGGPPS-T _{xpr2t} cassette in pUC-HUH-IntA	This study
pUC-HUH-IntA-SsGGPPS	P _{TEFin} -SsGGPPS-T _{xpr2t} cassette in pUC-HUH-IntA	This study
pUC-HUH-IntA-PaGGPPS	P _{TEFin} -PaGGPPS-T _{xpr2t} cassette in pUC-HUH-IntA	This study
pUC-HUH-IntA-SaGGPPS	P _{TEFin} -SaGGPPS-T _{xpr2t} cassette in pUC-HUH-IntA	This study
pUC-HUH-lip1-PaGGPPS	P _{TEFin} -PaGGPPS-T _{xpr2t} cassette in pUC-HUH-lip1	This study
pUC-Leu-A08-dSsLPPS _{RIDD}	P _{TEFin} -dSsLPPS _{RIDD} -T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-Leu-A08-dSsLPPS _{RIAD}	P _{TEFin} -dSsLPPS _{RIAD} -T _{xpr2t} cassette in pUC-Leu-A08	This study
pUC-HUH-IntE2-SsSCS _{RIDD}	P _{TEFin} -SsSCS _{RIDD} -T _{mig1t} cassette in pUC-HUH-IntE2	This study
pUC-HUH-IntE2-SsSCS _{RIAD}	P _{TEFin} -SsSCS _{RIAD} -T _{mig1t} cassette in pUC-HUH-IntE2	This study

Supplementary Table 2. Primers used in this study.

Names	Sequences (5' > 3')
A08-CcCLS-F	CAGCACTTTTTGCAGTACTAACCGCAGGCTTTCACCTT
A08-CcCLS-R	AAGTTCCGTAGTTGGATCCTACGTATTAGACCACGGACTCAAACAG
A08-SsLPPS-F	CAGCACTTTTTGCAGTACTAACCGCAGACCTCCGTGAA
A08-SsLPPS-R	TCCGTAGTTGGATCCTACGTATTAACGACAGGTCGGAACAGG
IntE2-TEFin-F	ATTGACTGGAACAGCTTAAGAGACCGGGTTGGCGGC
SsSCS-TEFin-R	GACTGCGGTTAGTACTGCAAAAAGTGCTGGTCCGAT
SsSCS-F	TTGCAGTACTAACCGCAGTCCCTGGCTTTCAA
SsSCS-R	ACAAGTTCCGTAGTTGGATCTTAAAAAACGAAGGACTTCATGTC
SsSCS-xpr2t-F	AAGATCCAACACTACGGAAGTTGTGTTGATGTC
IntE2-xpr2t-R	TATCGCAATCCACACTTAATTAAGACACGGGCATCTCACTTGC
A08-dCcCLS-F	CAGCACTTTTTGCAGTACTAACCGCAGTGCTCCGCTCGACGAAC
A08-dCcCLS-R	AGTTCCGTAGTTGGATCCTACGTATTAGACCACGGACTCAAACAG
A08-dSsLPPS-F	CAGCACTTTTTGCAGTACTAACCGCAGGACGCTTCTCAGGCTT
A08-dSsLPPS-R	CCGTAGTTGGATCCTACGTATTAACGACAGGTCGGAACAGGACC
IntE2-TEFin-F	ATTGACTGGAACAGCTTAAGAGACCGGGTTGGCGGC
dSsSCS-TEFin-R	TTGCAGTACTAACCGCAGGCTAAGATGAAGGAGAAC
dSsSCS-F	CCTGCGGTTAGTACTGCAAAAAGTGCTGGTCCG
IntE2-xpr2t-R	TATCGCAATCCACACTTAATTAAGACACGGGCATCTCACTTGC
IntA-TEFin-F	CTGATTGACTGGAACAGCTTAATTAAGAGACCGGGTTGGCGGC
YIGGPPS-TEFin-R	CCTGCGGTTAGTACTGCAAAAAGTGCTGGTCCG

YIGGPPS-F	TTGCAGTACTAACCGCAGGATTATAACAGCGCGGATT
YIGGPPS-R	TAGTTGGATCTCACTGCGCATCCTCAAAGTAC
YIGGPPS-xpr2t-F	TGCGCAGTGAGATCCAACACTACGGAACCTTGTGTTG
IntA-xpr2t-R	CCAAAGCTTGGTACCTTAATTAAGACACGGGCATCTCACTTGC
IntA-TEFin-F	CTGATTGACTGGAACAGCTTAATTAAGAGACCGGGTTGGCGGC
PaGGPPS-TEFin-R	AGCTGCGGTTAGTACTGCAAAAAGTGCTGGT
PaGGPPS-F	TTGCAGTACTAACCGCAGCTCTCGACAGGTCTGAGT
PaGGPPS-R	TCCGTAGTTGGATCTCAATGATGGTGGTGGTATGG
PaGGPPS-xpr2t-F	CACCACCATCATTGAGATCCAACACTACGGAACCTTGTGTTG
IntA-xpr2t-R	CCAAAGCTTGGTACCTTAATTAAGACACGGGCATCTCACTTGC
IntA-TEFin-F	CTGATTGACTGGAACAGCTTAATTAAGAGACCGGGTTGGCGGC
SaGGPPS-TEFin-R	GACTGCGGTTAGTACTGCAAAAAGTGCTGGTTCG
SaGGPPS-F	TTGCAGTACTAACCGCAGTCTTACTTCGACAAC
SaGGPPS-R	GGATCTTACTTTCGTCTTCGGATGGTGA
SaGGPPS-xpr2t-F	CGAAGACGAAAGTAAGATCCAACACTACGGAACCTTGTGTTG
IntA-xpr2t-R	CCAAAGCTTGGTACCTTAATTAAGACACGGGCATCTCACTTGC
IntA-TEFin-F	CTGATTGACTGGAACAGCTTAATTAAGAGACCGGGTTGGCGGC
SsGGPPS-TEFin-R	CCTGCGGTTAGTACTGCAAAAAGTGCTGGTTCG
SsGGPPS-F	TTGCAGTACTAACCGCAGGTCGCTCAGAC
SsGGPPS-R	GTTGGATCTTAGTGCTGTCGTCGGTAATAAA
SsGGPPS-xpr2t-F	CGACAGCACTAAGATCCAACACTACGGAACCTTGTGTTG
IntA-xpr2t-R	CCAAAGCTTGGTACCTTAATTAAGACACGGGCATCTCACTTGC

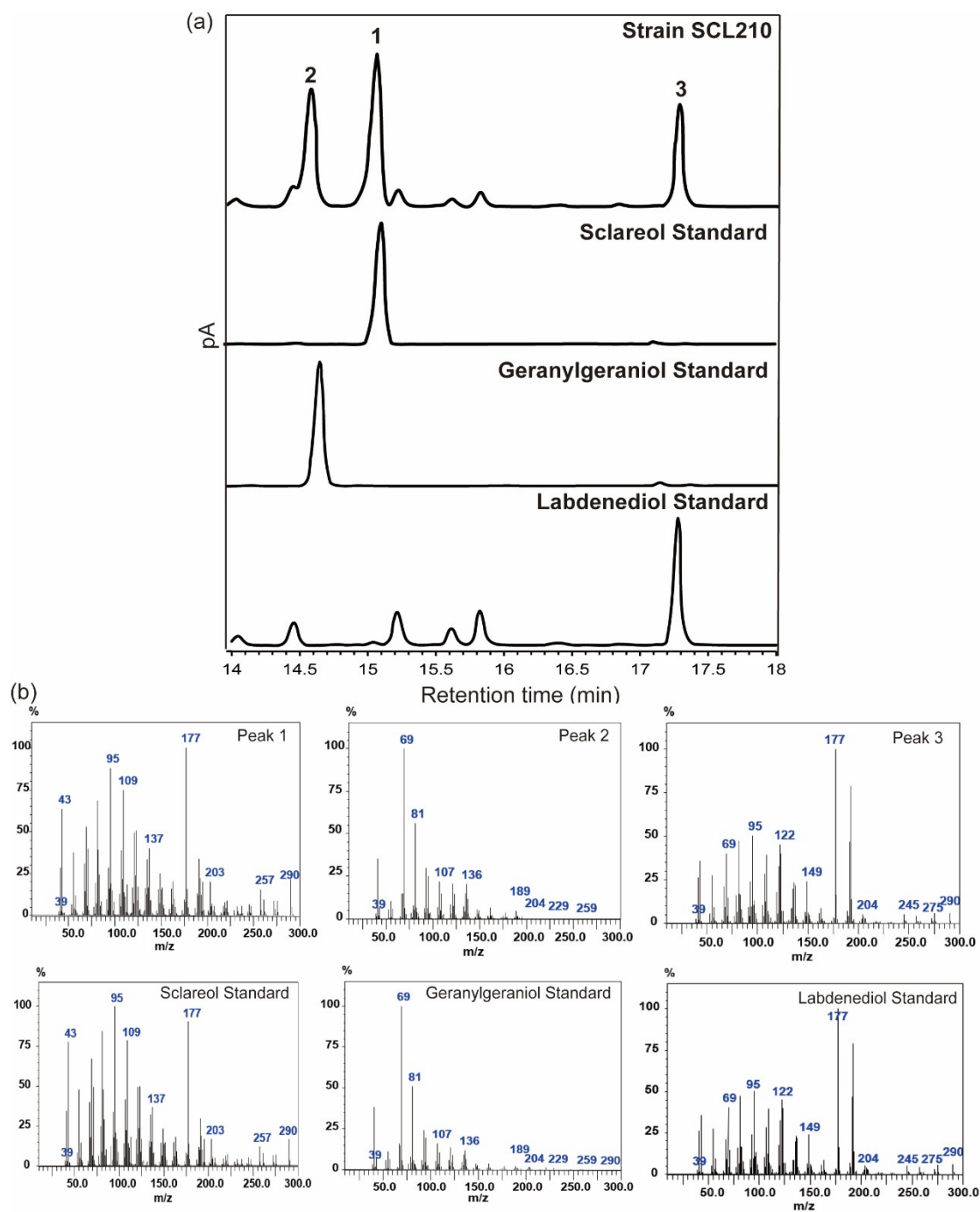
lip1Pa-TEFin-F	ATTGACTGGAACAGCTTAATTAAGAGACCGGGTTGGCGGC
lip1Pa-xpr2t-R	GTCGAACAAAAGCTTGGTACCTTAAGACACGGGCATCTCACTTG
TEFin-dSsLPPS-F	GCACTTTTTGCAGTACTAACCAGCAGGACGCTTCTCAGGCTTCT
RIDD-dSsLPPS-R	CCACCACCACCAACGACAGGTCCGAACAGGACCTTGAAGAT
dSsLPPS-RIDD-F	TGTCGTTGGTGGTGGTGGTTCTGGTGGTGGTGGCTCT
xpr2t-RIDD-R	AGTTCCGTAGTTGGATCCTACGTATTACTTGGCCTCCTCCTTCTCC
TEFin-dSsLPPS-F	CAGCACTTTTTGCAGTACTAACCAGCAGGACGCTTCTCAGGCTTC
xpr2t-RIAD- dSsLPPS-R	CCGTAGTTGGATCCTACGTATTAACAGCCCTCGGTGGCCTCCTTGATGATCTG ATCGGCCAGCTGATTGGCGTACTGCTCCAGACCACAACCGCCACCACCAGA
IntE2-TEFin-F	ATTGACTGGAACAGCTTAAGAGACCGGGTTGGCGGC
SsSCS-TEFin-R	GACTGCGGTTAGTACTGCAAAAAGTGCTGGTCCGAT
SsSCS-F	TTGCAGTACTAACCAGCAGTCCCTGGCTTTCAA
RIDD-SsSCS-R	GAACCACCACCACCAAAAACGAAGGACTTCATGTCCTC
SsSCS-RIDD-F	CTTCGTTTTTGGTGGTGGTGGTTCTGGTGGTGGTGGCTCTGGT
mig1t-RIDD-R	TCGACCGCCAGTGTTACTTGGCCTCCTCCTTCTCCAGTCGCTC
RIDD-mig1t-F	CCAAGTAACACTGGCCGGTCGATAATTTAACGTGC
IntE2-mig1t-R	CGCAATCCACACTTAATTA AAAA ACCCAA AAGGGCCGAAGGCTGG
IntE2-TEFin-F	ATTGACTGGAACAGCTTAAGAGACCGGGTTGGCGGC
mig1t-RIAD- SsSCS-R	TTAAATTATCGACCGCCAGTGTTAACAGCCCTCGGTGGCCTCCTTGATGATC TGATCGGCCAGCTGATTGGCGTACTGCTCCAGACCACAACCGCCACCACCAG AGCCAC
RIAD-mig1t-F	ACCGAGGGCTGTTAACACTGGCCGGTCGATAATTT
IntE2-mig1t-R	CGCAATCCACACTTAATTA AAAA ACCCAA AAGGGCCGAAGGCTGG

Supplementary Table 3. Synthetic gene fragment and gene sequences used in this study.

Names	Sequences (5' -> 3')
<p>Codon optimized (-)-8-hydroxy copalyl diphosphate synthase coding DNA (<i>CcCLS</i>) for <i>Y. lipolytica</i> based on the sequence of (-)-8-hydroxy copalyl diphosphate synthase (GenBank: ADJ93862.1) from <i>Cistus criticus</i></p>	<p>ATGGCTTTCACCTTACCTCCGCCACCTGTTCTGCCTGTCACCCGAGAACCCTCCGTCCACGT CAACTACTCCATCCCCCGGTAACCTGGCGACTCTGGTCTACCGCTAAGGGCGGTTCCAACAAG CTCGACATCCGACGACTCCGATGCTCCGCTCGACGAACCCCTGAGCCTTGGCTCAGGGTTCTA ACGGCGGTGAGACGGGTGTCGAGGCTATCCAGCGACTGCAGACCATCGCCGACGACAAGATCG ACGGCGGTGCTAACGAGCTGGGTATCGTCTGTTGGGACCTCATTTCGAGACGGCGTTCGACGCTGT CAAGTCTATGTTTACTCCATGGGCGACGAGACATCTCCATCTCCGCTACGACACCCGCTGG GTTGCTCTGGTTAAGGACGTGAACGGATCTGGCGGCCCCAGTTTCTTCTCTCCAGTGGAA TTGTGGACAACCAGCTGCCCGACGGCTCTGGGGTACTCTGAGGTTTTTCCGCTTACGACCG ACTGCTGAAGACCCTGGTTGTTGTCGCCCTCAAGTCTGGAACATCCGACCGACAAGTGC CAGAAGGGCCTGAAGTCTTTTCGAGACAACATTTCCAAGCTGGAGAAGGAGAACCCTCGAGGCC TCCGCTCAGATGCTGTCCGGTTTCGAGGTCGTCTCTCTGTCCTCATCGAGGTTGCCGACGACT CGACATCCAGATCCCCCTGCCATCCCTGTCTTTGAGGACCTGATTGCTCGACGAAACCTCAAG TTCGCCAAGATCCCCCTGGACCTGATGCACAACGTCCCCACCTCCCTGCTGAACCTCCCTGGAGG GTATGACCGCGCTCGAGCTGGACTGGGAGAAGCTGCTGAAGCTCCAGTCTCAGGACGGTTCT TTATCACTCCCTCCACCCGCTTCCGCTCTGATGCAGACCAACGACCAAGTGCAGTCCCTGGG CTACCTGAAGTTTGTGTTTCAAGAAGTTCAACGGCGGCGCCCGGTCAGTACCCTGTTGAGATT TTCGAGCGAATCTGGTCTGGACCGACTGCAGCGACTGGGTATCTCCCGTACTTCCAGTCCG AGATTAAGGAGTGTCTGGTACGCTTACGCTTCAAGCACTGGACCACTACCGCTCTCTGGGG TCGAAACACCCCTGTTTACGACCTGGACGACACCTGCATGGCCTTTAGAAATTTTGCAGTGCAC GGTACGAGCTGTCCGCTGAGGCTTTCGACACTTCGAGAAGAAGCGGCTCTTCTTCTGCTTCG GCTGGGAGACCCAGTACCTTACCTTAACTTCAACCTGTACCGAGCTTCCGCTTCTTCTGGG TCCCGGTGAGAACATTCTCAAGGAGGCTAAGCAGTTTTCTTAACTTCTCATGAAGAAGCAG GCCGCCCCGAGAGTCCAGGACAAGTGGGTTATCTGAAGGACTTCCCGGTGAGCTGAAGTAC GCCCTGGAGTTCCTGTTACGCTTCCGCTTCCGCTCGAGTGCAGACCCGATCTACGCTGAGCAGT ACGGTGGCGACAACGACGCTGATCGGCAAGACCCTTACCAGTGCCTACATCAACAACA ACGTCTACCTCGAGCTGGCAAGCTGGACTTAACTCAACTGCCAGGCCCTGACCCGAAAGT GGGAGACCATGCAGAAGTGGTTTATGAGGTCCAAAGCTGGACGAGTTCGCGCTCTTCTTAA CCCTGCTGGAGTCTACTTCTGCGCCGCTGCTTCCATTTTGGAGCCGAGCGATCTACCGAGCG ACTGGCTTGGGCTAAGACCGCTTTCCTCATGGAGACCATGGCTCCTACTTCGACGACGAGATG AACTCAAGGACCTGCGAAAGCCCTTCGTTCAAGGAGTTTAAAGAATCTACGACGACGACGAA GAGGCCAAGGGTACTAAGTGAACCTGATCATCATTCTGCTGACCACCTGAACCACCTCACC GAGGTCTGCGGACGAGACATCAACTCTACCTGTGCCACTCCTGGGAGAAGTGGATGATGATG TGGGAGCCGAGGGCGACCGATACAAGGGTGTGCTGCTGAGCTGCTGCAACTCATCAACTG TCTTCCGACGACTGTTTTCAACGACACCCTGTCCACCCCAACTACGAGAAGCTGGTGACCC TGTTCAACAAGCTGTGCCACCAGCTGGGCACTCCCGACGAGGTAACCAACAGGAGGACTCTG ACATCAAGGACACCAAGATCGAGATCGCCATGCAAGGAGTGGTCCAGCTGGTCCACCAAGT CCTCCGACGACATCTCCATGGACCTGAAGCAGACCTTCTTCGCCGTGGTGGATCCTTCTACTA CGCCGCTACTGCGACCGAGGCCACCATTAACCTCCACATCGTTAAGGTCCTGTTTGGAGTCCGT GTCTAA</p>
<p>Codon optimized (13E)-8α-hydroxylabden-15-yl diphosphate synthase coding DNA (<i>SsSCS</i>) for <i>Y. lipolytica</i> based on the sequence of (13E)-8α-hydroxylabden-15-yl diphosphate synthase (GenBank: AFU61897.1) from <i>Salvia sclarea</i></p>	<p>ATGACCTCCGTGAACCTGTCGCGAGCCCTGCTGCTATCATCCGACGACGACTGCAGCTGCAGC CCGAGTTTACGCTGAGTGTCTGGCTGAAGTCTCTCCCAAGACGCCCCCTTACCCTTCTCT TGCCAGATCCGACCCAAGCAGCTCTCCAGATTGCCGAGCTGCGAGTACCTCCCTGGACGCTT CTCAGGCTTCTGAGAAGGACATCTCCCTCGTCCAGACCCCAAGGTCGAGGTTAACGAGAGA AGATCGAGGAGTCCATCGATCGTTCAGAACCTGCTGATGACCTCCGGAGGAGCCCGAATTT CCGTCTCCCTTACGACACCCCGCTCATCGCTCTCATCAAGGACCTGAAGGGCCGAGACGCC TCAGTTTCTTCTGCTGCTGAGTGGATCGCCACCACCAGCTGGCTGACCGGTTCTTGGGGTAC GAGTTTTCTGTATTTACGACCGAATCTCGAACCCCTGGTTGCTGCTGCCCTCAAGTCTTTG GAACCTCCAGTCCGACATCATCGAGAAGGGCGTACCTACATCAAGGAGAACGTGCACAAGCT GAAGGGCGCCAACGTGGAGCAGCAAGCCGCTGGTTTTGAGCTGGTCTGCCACCTCATGCA GATGGCCACCGACTGGCATTACGGACTCCCTTACGACCCCTCATCAAGGAGTACGCG CGACACCAAGAAGCAGCGACTGAAGGAGATCCCCAAGGACCTCGTCTACCAGATGCCACCAA CCTGCTGTACTCCCTCGAGGGTCTCGGCGACCTGGAGTGGGAGCGACTGCTGAAGCTGCAGTCC GGAAACGGCTCCTTCCGACTCCCTTCCCTCCACCGCTGCTGTTCTCATGCAACCAAGGACG AGAAGTGTCTGAAGTACATTGAGAACGCTCTGAAGAAGTGCAGCGGCGGCTCCTCACACCT ACCCTGTTGACATCTTCTCCGACTGTGGGCCATCGACCGACTGCAGGACTGGGTATCTCCCG ATTCTTCCAGCAGGATTAAGTACTTCTGGACACATCGAGTCCGCTGGGAGGAGACCCG AGTCTTTTCTGGCCGATACACCAAGTTTTCCGACATCGACGACACCTCCATGGCGTCCGACTC CTGAAGATGCACGGTACGACGCTGACCCCAACGTCCTGAAGCACTTCAAGCAGCAGGACCGG AAGTTTTCTGCTACATCGGCCAGTCCGTGGAGTCCGCTTCCCTATGTACAACCTGTACCGAG CCGCCAGCTCCGATTCCCTGGTGGAGGTTTTTCGAGGAGGCCACCAAGTTTGCCTTAACTT TCTGCAGGAGATGCTGGTCAAGGACCGACTGCAGGAGCGATGGGTGATCTCCGACCACTCTT TGACGAGATCAAGCTGGCCTGAAGATGCCCTGGTACGCCACCCTGACTGAGTTGAGGCTGC TTACTACCTGGACACTACGCCGCTCCGGTGACGTTTTGGATCGGTAAGTCTTCTACCAGATG CCCGAGATCTCAACGACACTACAAGGAGCTGGCCATTCTGGACTTCAACCGATGCCAGACTC CAGCACCAGCTGGAGTACATTCACATGAGGAGTGGTACGACCGATGCTCCCTGTCAGGATTC GGCATCTCAAGCGAGAGCTGCTGCGATCTACTTCTGGCCGCTGCTACCATCTTTGAGCCCG AGCGAACCCAGGAGCAGTCTGTGGTAAGACCCGAATTTTGCCAAAGATGATCACTCCCTT CGTCAACATCTCCGGCACCACCTGTCCCTGGACTACAACCTCAACCGCTGGACGATCATC TCTCCGCCAACGAGGACCGGCTGGTGGTACTCTGCTGGTACTTCCACCAGCTGCTGG ACGGTTTTCGACATCTACCCCTGCACCAGCTCAAGCAGCTGCTGGTCCCGATGGTTCATGAAGGT CCAGCAGGGCGAGGGCTCTGGTGGTGGAGGACGCTGTTCTGCTCGCAACACCGCTGAACATG CGCCGGTCTGAACGAGGACGCTCTGTCTAACAACGAGTACACCGCCCTGTCCACCCTACCAAC AAGATTTGCAACCGACTGGCCAGATCCAGGACAACAAGATCTCTCAGGTCTGCGACGGGTCC ATCAAGGACAAGGAGCTGGAGCAGGACATGCAGGCCCTCGTTAAGCTGGTCTGAGGAGAAC GGCGGGCTGTTGACCGAAACATTCGACACACCTTCTCTCCGTTCTCAAGACCTTCTACTACG ACGCTTACCACGACGACGAGACCAGCCGACTGCACATCTTCAAGGTCCTGTTCCGACCTGCTGC TTAA</p>

<p>Codon optimized sclareol synthase coding DNA (<i>SsScS</i>) for <i>Y. lipolytica</i> based on the sequence of sclareol synthase (GenBank: AFU61898.1) from <i>Salvia sclarea</i></p>	<p>ATGTCCCTGGCTTTCAACGTCGGCGTCAACCCCTTCTCCGGCCAGCGAGTTGGTTCCCGAAAGG AGAATTCCCCGTCCAGGGCTTCCCCGTTACCACCCTAACCCGATCCCGACTGATCGTCAACTG TTCCCTGACCACCACGACTTCATGGCTAAGATGAAGGAGAACTTTAAGCGGAGAGCGACAAA GTCCCCACCACCACCCTGCGATTCGGAGGACATCCCTTCCAACCTGTGCATCATCGACACC CTGCAGCGACTGGGCGTCGACCAATTTTTTTCAGTACGAGATCAACACCATCCCTCGACAACACT TTCCGACTGTGGCAGGAGAACACAAAGTTCATCTACGGCAACGTCAACCACCACCGCATGGCTT TCCGACTGCTGCGAGTCAAGGGCTACGAGGTCCTCCGAGGAGCTGGCTCCTACCGTAACCA GGAGGCCGCTCCCAGCAGACCAACGACCTGCCTATGATCATCGAGCTGTACCAGCCGCCAA CGAGCGAATCTACGAGGAGGAGCGATGCCCTGGAGAAGATTCTGCGCTGGACCACCATTTTTCT CAACAAGCAGGTCCAGGACAACCTCATCCCCGACAAGAAGCTGCACAAGCTGGTCGAGTTTTA CCTGCGAACTACAAGGCATCACCATCCGACTCGGTGCTCGACGAAACCTGGAGCTGTACGA CATGACCTACTACCAGGCCCTGAAGTCCACCAACCGATTCTCCAACCTGTGTAACGAGGACCTC CTGGTCTTCGCCAAGCAGGACTTCGACATCCACGAGGCCAGAACAGAGGACTGCAGCAG CTGCAGCGATGGTACGCTGACTGCCGACTGGACACCCTGAACCTCGGCCGAGCAGCTGTGATC GTTGCCAACTACCTGGCTTCCCTGATTATCGGGGACCACGCTTCGACTACGTCACCGACTGGCTT CGCCAAGACCTCCGTTCTGGTACCATCATGGACGACTTTTTTACTGCCACGGCTCCTCCAG GAGTGTGACAAGATCATCGAGCTGTCAAGGAGTGAAGGAGAACCCGACGCTGAGTACGG CTCCGAGGAGCTCGAGATCCTGTTTTATGGCCCTGTACAACACCGTCAACGAGCTGGCCGAGC AGCTCGAGTTGAGCAGGCTCGATCCGTCAGGAGTTCCTGGTGAAGCTGTGGGTGGAGATCCT GTCCGCCTTCAAGATCGAGCTGGACACCTGGTCCAACGGCACCCAGCAGTCTTTTGACGAGTAC ATCTCCTTCTCCGCTGTCCAACGGCTCCCGACTCACCGGTCTGCTGACCATGACGATTCGTCGG CGTCAAGCTCTCCGACGAGATGCTCATGTCCGAGGAGTGCACCCGACTGGCTCGACACGTTTGC ATGGTCCGGCGACTGCTGAACGACGCTGTGCTTCCGAGCGAGAGCGAGGAGGAGAATCGCC GGTAAGTCTACTCCATCCTCTGGCCACCGAGAAGGACGGTCAAGGAGTCCGAGGACGAG GCTATCGCCGAGATTAACGAGATGGTTCGAGTACCCTGGCGAAAGGCTCTGCAGATCGTCTAC AAGAAGGAGTCCATCCTGCCCGACGATGTAAGGACGCTTTCTGGAGATGGCCAAGGGCACC TTCTACGCTACGGCATTAAACGACGAGCTGACCTCCCCCAGCAGCTAAGGAGGACATGAAG TCCTTCGTTTTTAA</p>
<p>Endogenous geranylgeranyl diphosphate synthase (<i>YIGPPS</i>) coding DNA (<i>GGS1</i>, YALI0D17050g)</p>	<p>TAGGATTATAACAGCGCGATTTCGAAGGAGATATGGGGCAAGGCCGCCGACCCGCGCTGCTG GGACCGTACAACTACCTCGCCAAACACCGGGGCCACAACATCAGAGAACAATTGATCGCAGCG TTCGGAGCGGTTATCAAGGTGGACAAGAGCGATCTCGAGACCATTTCCGACATCACAAGATT TTGCATAACTCGTCGCTGCTTGGTATGACGTGGAAAGACAACCTGATGCTCCGACGAGGCTGC CGGCAGCCATTGCTGTTTGGAGTCCCCAAACCATCAACTCCGCCAACTACATGACTTTGT GGCTCTGCAGGAGGTGCTCAAGTCAAGTCTTATGATGCCGTCTCCATTTTACCAGGAAATG ATCAACTTGCATAGAGGTCAGGGATGGATCTTACTGGAGAGAAACACTACTTCCGCTCCG AAGACGAGTATCTGGAGATGGTGGTGCACAAGACCGGTGGACTGTTTCCGGCTGGCTCTGAGC TTATGCTGTCCGTTGGCATGAAACAGGAGGACCATGAAAAGATCAACTTTGATCTCACACCC TTACCGACACACTGGGAGTCAATTAACGATTCTGGATGATTACCTCAACCTCCAGTCCACCGGA ATTGACCGAGAACAAGGGATTCTCGGAAGATATCAGCGAAGGAAAGTTTTCGTTCCCGCTGAT TCACAGCATACGCCAACCCGGATAACCCAGGAGATTCTCAACATTCTCAAACAGCGAACAAG CGACGCTTCACTCAAAAAGTACCGCTGGACTACATGAGAACAGAAACCAAGAGTTTTCGACTA CTGCCTCAAGAGGATACAGGCCATGCTCACTCAAGGCAAGTTCGTACATTGATGATCTAGCAGC AGCTGGCCACGATGCTCCAAGTACGAGCATTTTGCATTATTTTGTGCCACCTCTGACTGTG AGGAGAGAAAAGTACTTTGAGGATGCCGAGTGA</p>
<p>Codon optimized geranylgeranyl diphosphate synthase (<i>SsGGPPS</i>) for <i>Y. lipolytica</i> based on the geranylgeranyl diphosphate synthase (<i>GGPPS7</i>, GenBank: WP011429285.1) from <i>Synechococcus</i> sp.</p>	<p>ATGGTCCGCTCAGACCTTCAACCTGGACACCTACCTGTCCCAGCGACAGCAGCAGGTTGAG GAGGCTCTGTCTGCTGCTCTGGTCCCTGCTTACCCTGAGCGAATCTACGAGGCCATGCGATACT CCCTCCTGGCTGGTGGTAAGCGACTGCGACCTATCCTCTGCCTCGCTGCTTGTGAGCTGGCTGG TGGCTCTGTTGAGCAGGCTATGCCTACCCTGCTGCTGAGATGATTCACACCATGTCTCTG ATCCACGACGACCTCCCGCTATGGACAACGACGACTTTCGACGAGGCAAGCCTACCAACCAC AAGTCTTCGGCGAGGACATCGCCATTCTGGCTGGTACGCTCTGCTGGCTTACGCTTTCGAGC ACATCGCCTCCAGACCCGAGGTGTTCCCTCCTCAGCTGGTCTCCAGGTCATCGCTCGAATCCGG TCACGCTGTGCTGCTACCGGCTGCTGGTGGTCAAGTTGTCGACCTGGAGTCCGAGGGTAAAG GCTATCTCCCTGGAGACCCTCGAGTACATTCACCTCCACAAGACCGGCCCTGCTGGAGGCTT CTGTTGTTTCTGGCGGTATCCTGGCCGGCTGACGAGGAGCTGTGGCTCGACTGTCTCACTA CGCTCGAGACATTGGCCTGCCCTTTCAGATCGTCGACGACATTCGGACGCTGACCTACCTCT GAGCAGCTGGTAAGACCCTGGTAAGGACCAGGCTGCTGTAAGGCTACCTACCTTCCCTG CTGGGTCTGGAGGCTTCCCGACAGAAGGCTGAGGAGCTGATTAGTCCGCCAAGGAGGCTCTG CGACCTTACGGTTTCAGGCTGAGCCTCTCCTGGCTCTGGCTGACTTATTACCAGCAGCAGC ACTAA</p>
<p>Codon optimized geranylgeranyl diphosphate synthase (<i>PaGGPPS</i>) for <i>Y.</i> <i>lipolytica</i> based on the 390-719 AA of</p>	<p>ATGCTCTCGACAGGCTGAGTCTCTCCAGTGCATTCCAACGAGGGCAAGGATCTTCAGCGAG TGGACACCGACCACATTTTCTTGGAGAAGGCTGTTTTGGAGGCTCCTTACGACTACATTGCCTC CATGCCTTCCAAGGGTGTTCGTGATCAGTTTCAICGACGCTCTCAACGACTGGCTGCGGGTGCCC GACGTCAAGGTTCGGTAAGATCAAGGACGCGCTGCGCGTACTGCATAAATAGCTCTACTTCTGG ATGACTTCCAGGACAACCTCGCTCTCCGTAAGGAAAGCCCTCCACCACAACATCTTCGGATC AGCCCAGACGGTCAACACCGCCACTTATAGTATCATAAAGGCCATCGGCCAGATCATGGAGTT TTCAGCTGGAGAGTCTGTCAGGAGGTCATGAACCTCATCATGATTCTCTTTTCAAGGACAGGCC ATGGACCTGTTCTGACCTACAACGGCCACGTTCCCTCTGAGGAGGAGTACTACCGAATGATTG ACCAGAAAACCTGGCCAACCTGTTTTCAATTGCAACTTCTTCTGTCTCAATGCTGCCGATAACGA AATCCCGCGAACGAAAAATCCAGAGGCTTTTACATCGACTGACACCGCTACTTGGAAAGATGCTT CCAGATTTCGAGACGACTACCAGAACCTGGTTTCTGCTGATTACCAAGCAGAAGGGCTTCTGC GAGGATCTCGATGAGGGGAAGTGGTGGCTGGCGCTCATTACATGATCCACAAGCAACGGGAC CATATGGCGTTGTTAATGTGCTTTCAACAGGACGAAAAGCACGGCTGATGATGACCTCGAGCA AAACAGTTTGTGCTGGACATTATCGAGGAAGAAAAGTCCCTCGATTATACTAGGTCTGTGATGA</p>

<p>Fusicoccadiene Synthase (GenBank: A2PZA5.1) from <i>Phomopsis amygdal</i></p>	<p>TGGATCTGCACGTGCAGCTGAGAGCCGAAATTGGCCGATTGAGATTCTGTTGGACTCGCCCAA CCCCGCAATGCGACTCTGTGGAACTGCTGCGAGTCCATCACCACCACCATCATTGA</p>
<p>Codon optimized geranylgeranyl diphosphate synthase (<i>SaGGPPS</i>) for <i>Y. lipolytica</i> based on geranylgeranyl diphosphate synthase (GenBank: BAA43200.1) from <i>Sulfolobus acidocaldarius</i></p>	<p>ATGTCTTACTTCGACAACACTTCAACGAGATCGTGAACCTGTGAACGACATCATCAAGTCTT ACATCTCTGGCGACGTGCCAAGCTGTACGAGGCCTTTACCACCTGTTACCTCTGGCGGCAA GCGACTGCGACCCCTGATCCTGACCATCTTTCTGACCTGTTCCGGCGGACAGCGAGAGCGAGCC TACTACGCCGGCGCCGCCATCGAGGTGCTGCACACCTTACCCTGGTGCACGACGACATCATGG ACCAAGACAACATCCGACGAGGCCTGCCACCCTGCACGTGAAGTATGGCCTGCCTCTGGCTA TTCTGGCCGGCGATCTGCTGCACGCCAAGGCCTTTCAGCTGCTGACCCAAGCCCTGCGAGGCCT GCCCTCTGAAACCATCATCAAGGCCTTCGACATCTTACCCTGATCTATCATTATCATCTCTGAG GGCCAAGCCGTGGACATGGAGTTCGAGGACCGAATCGACATCAAGGAGCAAGAGTACCTGGA CATGATCTCTCGAAAAGACCCGCCCTGTCTCTGCCTTCTCTATCGGGCCCTGATCGCCG GCGCCAACGACAACGACGTGCGACTGATGTCTGACTTCGGCACCAACCTGGGCATCGCCTTCA GATCGTGGACGACATCTGGCCTGACCGCCGACGAGAAGGAGCTGGGCAAGCCCGTGTCTC TGACATCCGAGAGGGCAAGAAGACCATCCTGGTATCAAGACCCCTGGAGCTGTGTAAGGAGGA CGAGAAGAAGATCGTGTGAAGGCCCTGGGCAACAAGTCTGCCTTAAAGGAGGAGCTGATGTC TTCTGCCGACATCAAGAAGTACTCTTGGACTACGCTACAACCTGGCCGAGAAGTACTAC AAGAACGCCATCGACTCTGTAACCAAGTGTCTTCTAAGTCTGACATCCCCGGCAAGGCCCTGA AGTACCTGGCCGAGTTCACCATCGAAGACGAAAGTAA</p>
<p>Codon optimized linker (GGGS)₃ and peptide RIDD for <i>Y. lipolytica</i> based on reference²</p>	<p>GGTGGTGGTGGTCTGGTGGTGGTGGCTCTGGTGGTGGCGGTTGTGGTTCTCTGCGAGAGTGTG AGCTGTACGTGCAGAAGCACAACATTCAGGCTCTGCTGAAGGACTTATTGTGCAGCTGTGTAC CGCTCGACCTGAGCGACCCATGGCTTTTCTGCGAGAGTACTTTGAGCGACTGGAGAAGGAGGA GGCCAAG</p>
<p>Codon optimized linker (GGGS)₃ and peptide RIAD for <i>Y. lipolytica</i> based on reference²</p>	<p>GGAGGCGGCGGATCTGGCGGAGGAGGCTCTGGAGGCGGAGGATGCGGCCCTGGAGCAGTACGC CAATCAGCTGGCCGATCAGATCATCAAGGAGGCCACCGAGGCTGT</p>
<p>TEFin Promoter</p>	<p>AGAGACCGGGTGGCGGCGCATTGTGTCCAAAAAACAGCCCAATTGCCCAATTGACCCC AAATTGACCCAGTAGCGGGCCAAACCCCGCGAGAGCCCTTACCCACATATCAAACCTC CCCCGGTCCCACACTTGGCGTTAAGGGCGTAGGGTACTGCAGTCTGGAATCTACCGTTGTTC GACTTTGTAAGTACTTCTTGTCTGGCCATCCGGGTAACCCATGCCGACGCAAAAATAGACTAC TGAAAATTTTTTGTCTTGTGGTTGGGACTTTAGCCAAGGGTATAAAAAGACCACCGTCCCCGAA TTACCTTTCTCTTCTTCT CTGAGTATAAGAATCATTCAAAATGGTGAGTTTCAGAGGCAGCAGCAATTGCCACGGGCTTG AGCACACGGCCGGGTGTGGTCCCATTCCCATCGACACAAGACGCCACGTCATCCGACCAGCAC TTTTTGCAGTACTAACCAG</p>
<p>xpr2t Terminator</p>	<p>GATCCAACACTACGGAACCTGTGTGTGATGTCTTTGCCCGGCTCCGATATCATCTCTGCCTCTTAC CAGTCCGACTCTGGTACTTTGGTCTACTCCGGTACCTCCATGGCCTGTCCCCACGTTGCCGGTCT TGCTCCTACTACCTGTCCATCAATGACGAGGTTCTCACCCTGCCAGGTCGAGGCTCTTATTA CTGAGTCCAACACCGGTGTTCTTCCACCACCAACCTCAAGGCTCTCCCAACGCTGTGGCTA CAACGGTGTGGCATTAGGCAATTAACAGATAGTTTGGCCGGTGTATAATCTCTTAACTCCCA CACTCCTTTGACATAACGATTATATGTAACGAAACTGAAAATTTGACCAGATATTGTTGTAATAAG AAAATCTGGCTTGTAGGTGGCAAAATGCGGCTCTTTGTTTCAATTCCTCTGTGACTACTC GTCATCCCTTATGTTGACTGTCTGATTTCTTATTTCCATACATATGCAAGTGAGATGCCGT GTC</p>
<p>mig1t Terminator</p>	<p>CACTGGCCGGTCGATAAATTTAACGTGCTGAGCTCAGCACACGCATTGCCATTGGCTGTATATA GATGAATGTAATGATACCGTAAGAGAATGAGAGCACGGTATTGTATTACAGGGGATTAAGTAC ACATTACTTGGAGTCTGTACCAGAAGACTACTATACATGGTATTACTTACATTAGAGTCCG TGACCGTATTCTGCTCGTATAGACATAAATTTTCCCTACCCACATTTGTTCTGGGCCCTCCGGAG CACATCTACAGTGAGTACTGTTTTCAGTTGAGCTTGAGGGGTTAAGTAAGTGGGGGAAGGGTT TGGGATTCTGAAAAGAGCATGACTAATCTCTCTGTGGAGGAGCAATGAAGTACGCTGATGCA ATCATACCGGTGTATCGGATCTGCCTGGGTGTCTGATTACTAATCATTACTACACTGTTTCC CAGCTATCTCATCATCTCAGAGCCTCGGCCAGCCTTCGGCCCTTTGGGTTT</p>



Supplementary Figure 1. GC-MS analysis of the diterpenoid standards and the products generated by strain SCL210. (a) Gas chromatogram of dodecane phase obtained from strain SCL210 culture after 120 h fermentation and gas chromatogram of sclareol standard (retention time 15.1 min), geranylgeraniol (GGOH) standard (retention time 14.6 min), labdenediol (LOH) standard (retention time 17.3 min). (b) The mass spectrum of peak 1-3 and the diterpenoid standards.

References

1. Y. Ma, W. Li, J. Mai, J. Wang, Y. Wei, R. Ledesma-Amaro and X.-J. Ji, *Green Chem.*, 2021, **23**, 780-787.
2. W. Kang, T. Ma, M. Liu, J. Qu, Z. Liu, H. Zhang, B. Shi, S. Fu, J. Ma, L. T. F. Lai, S. He, J. Qu, S. Wing-Ngor Au, B. Ho Kang, W. C. Yu Lau, Z. Deng, J. Xia and T. Liu, *Nat. Commun.*, 2019, **10**, 4248.