Supporting Information

Efficient Biosynthesis of R-(-)-linalool through Adjusting Expression Strategy and Increasing GPP Supply in *Escherichia coli*

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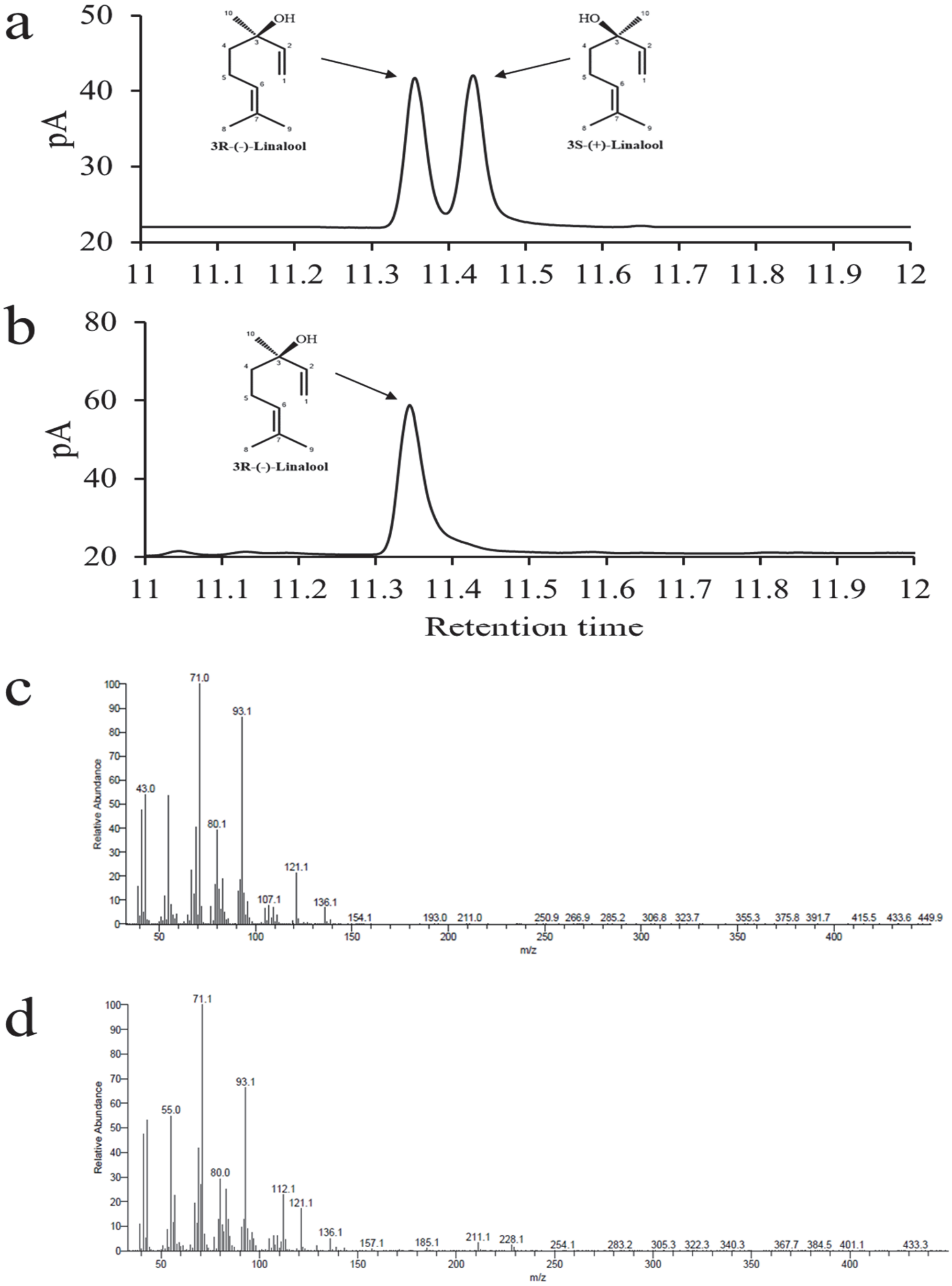

Figure S1. Chiral phase GC and GC-MS identification of R-(-)-linalool product of LIS. (a) chiral phase GC analysis of an authentic linalool standard, the left and right peaks correspond to R-(-)-linalool and S-(+)-linalool, respectively. (b) chiral phase GC analysis of products from recombinant strain WX6000 (c) Mass spectrum of an authentic linalool standard. (d) Mass spectrum of the peak at retention time of 13.3.

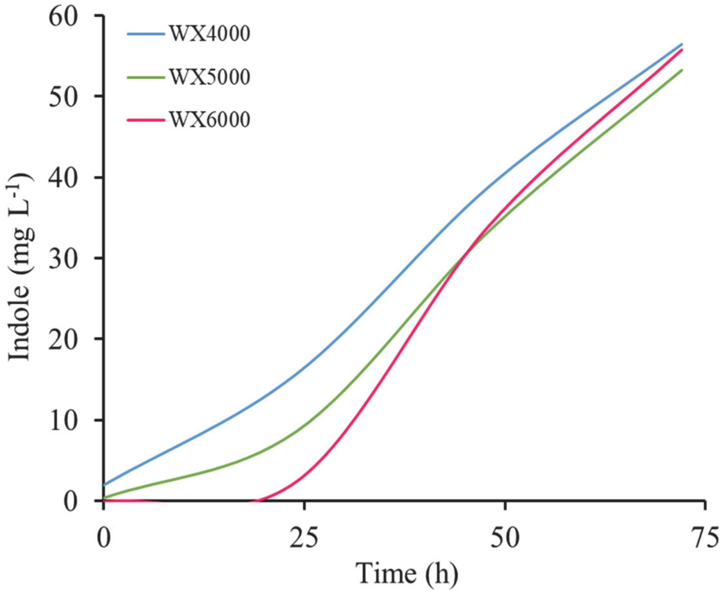

Figure S2. Formation of R-(-)-linalool was accompanied by production of approximately 50 mg/L indole.

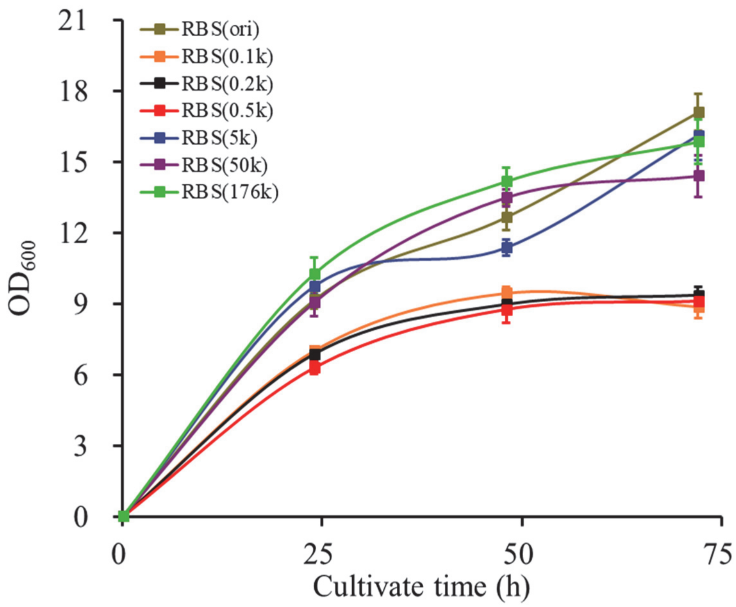

Figure S3. OD600 for recombinant strains containing different bLIS TIR strengths.

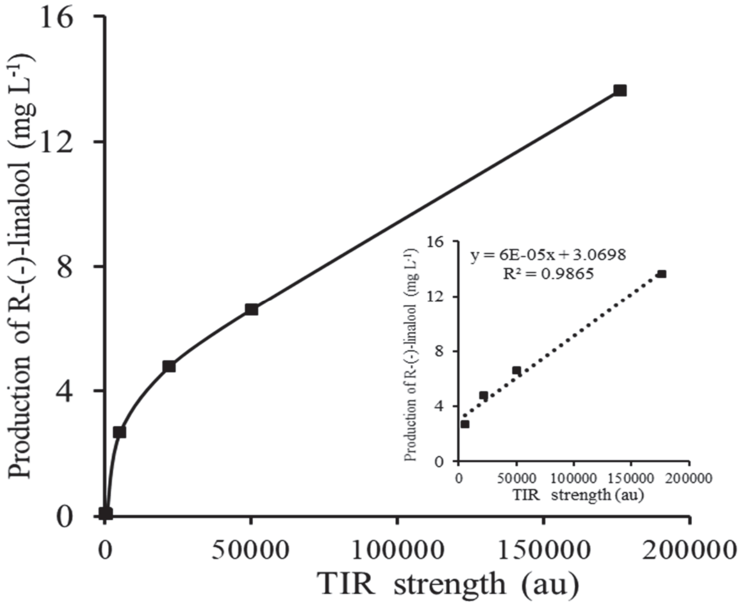

Figure S4. Positive correlation between R-(-)-linalool production and bLIS expression level, especially when the TIR strength of bLIS is between 5k au and theoretical maximum 176k au.

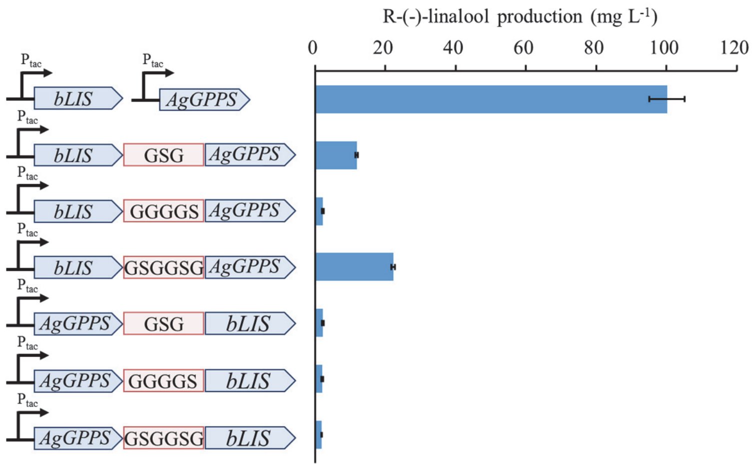

Figure S5. Microbial production of R-(-)-linalool via fusion of AgGPPS and bLIS, forward and reverse fusion of bLIS and AgGPPS through three short flexible linker GSG, GGGGS and GSGGSG, respectively, samples were measured at 72 h.

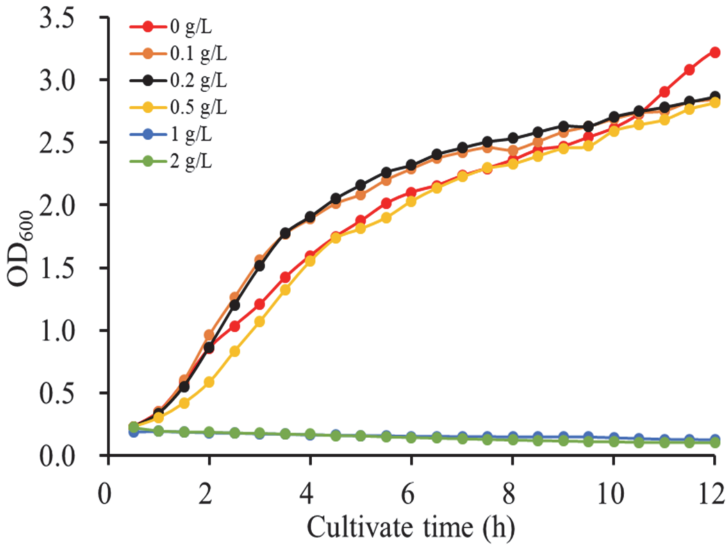

Figure S6. OD_{600} value of *E. coli* CIBTS1758 which were cultivated in LB medium containing different concentration of R-(-)-linalool for 12 h, samples were automatically measured each 0.5 h.

Table S1. Primers used in this study

Name	Sequence 5' → 3'
MK-F	CGAGCTCCTGCATAAAGGAGGTAAAAAAACATGG
MK-R	AACTGCAGTTAATCTACTTTCAGACCTTGCTCGGT
RBS0.1K-F	TAATATTCCCGCTACAGTATCCATGGGCATG
RBS0.1K -F	CTACAAATTCTGGTGTGAAATTGTTATCCGCTCA
RBS0.2K-F	GTAACTATACCAGGCAGTATCCATGGGCAT
RBS0.2K -R	TCGTGTAGCCGAAGTGTGAAATTGTTATCCG
RBS0.5K -F	AGGCACCGCTCCACAGTATCCATGGGCATGCAGG
RBS0.5K -R	TCGTTGTCGTGTGAAATTGTTATCCGCTCACAAT
RBS5K-F	GCACGGGAGGTAGGCAGTATCCATGGGCATGCAGG
RBS5K-R	GGATAATCGTGTGAAATTGTTATCCGCTCACAAT
RBS50K-F	AAGGAGTAGTACAGTATCCATGGGCATGCAGG
RBS50K-R	ACTTCTCGTGTGAAATTGTTATCCGCTCACAAT
RBS176K-F	AACGATAAGGAGGTTTTTTATGGGCATGCAGGAATTTGAA
RBS176K-R	TACTTCCGTACTTGTATTGTTATCCGCTCACAATTCCAC
Fusion tags-F	CAGGAATTTGAATTTGCCGTTC
Fusion tags-R	GGATACTGTTTCCTGTGTGAAATTG
MBP-F	tcacacaggaaacagtatccATGAAAATCGAAGAAGGTAAACTGG
MBP-R	acggcaaattcaaattcctgAGTCTGCGCGTCTTTCAGGG
NusA-F	tcacacaggaaacagtatccATGAACAAAGAAATTTTGGCTGTAGT
NusA-R	acggcaaattcaaattcctgCGCTTCGTCACCGAACCA
CmR29-F	tcacacaggaaacagtatccATGGAGAAAAAAATCACTGGATATACC
Cm29R-R	acggcaaattcaaattcctgAGCAACTGACTGAAATGCCTCA
GST-F	tcacacaggaaacagtatccATGTCCCCTATACTAGGTTATTGGAAA
GST-R	acggcaaattcaaattcctgTTTTGGAGGATGGTCGCCA
GPPS plasmid vector-F	AGATCTCAATTGGATATCGGCCG
GPPS plasmid vector-R	ATGTACTGTTTCCTGTGTGAAATTGTT
IspA-F	tcacacaggaaacagtacatATGGACTTTCCGCAGCAACTC
IspA-R	ccgatatccaattgagatctTATTTATTACGCTGGATGATGTAGTCC
IspA(S80F)-F	TTAATTCATGATGATTTACCGGCAATG
IspA(S80F)-R	AAAGTAAGCGTGGATACACTCAACGG
Erg20-F	tcacacaggaaacagtacatATGGCAAGTGAAAAAGAAATTCG
Erg20-R	ccgatatccaattgagatctTTATTTGCTACGCTTATACACCTTGTT
Erg20(F96W)-F	TTAGTTGCCGATGATATGATGGATAA
Erg20(F96W)-R	CCAATAAGCCTGCAACAGTTCG
Erg20(N127W)-F	GATGCGTTTATGCTGGAAGCAG
Erg20(N127W)-R	CCAGATGGCAATTTCGCCC

Table S2. Plasmids and strains used in this study

Name	Relevant characteristics	References
Plasmids		
pHGFH	Ptac *ispH*$_{AS}$ *ispG*$_{TE}$ *petF*$_{TE}$ *petH*$_{TE}$, pSU2718 ori, Cm^r	[S1]
pAGES	PGI* *fldA ispG*, Ptrc *mvaE*$_{EF}$ *mvaS*$_{EF}$, pCL1920 ori, Spec^r	[S1]
pETDuet-1	pBR322 origin; Amp^r; Double T7 promotors	Novagen
pETDuet-tac	The T7 promotors of pETDuet-1 are placed by tac promotors	This work
pETDuet-*laLIS*	pETDuet-tac carrying *L. angustifolia laLIS*	This work
pETDuet-*ofLIS*	pETDuet-tac carrying *O. fragrans var. thunbergii ofLIS*	This work
pETDuet-*bLIS*	pETDuet-tac carrying *S. clavuligerus bLIS*	This work
pETDuet-*bLIS-MK*	pETDuet-*bLIS* carring *Methanosarcina mazei MK* at second multiple cloning site	This work
pETDuet-*bLIS* (0.1k)	pETDuet-tac containing *bLIS* with TIR of 100	This work
pETDuet-*bLIS* (0.2k)	pETDuet-tac containing *bLIS* with TIR of 200	This work
pETDuet-*bLIS* (0.5k)	pETDuet-tac containing *bLIS* with TIR of 500	This work
pETDuet-*bLIS* (5k)	pETDuet-tac containing *bLIS* with TIR of 5000	This work
pETDuet-*bLIS* (50k)	pETDuet-tac containing *bLIS* with TIR of 50000	This work
pETDuet-*bLIS* (max)	pETDuet-tac containing *bLIS* with TIR of 176000	This work
pETDuet-*MBP*bLIS*	pETDuet-tac carrying *MBP* fused to N-terminus of *bLIS*	This work
pETDuet-*NusA*bLIS*	pETDuet-tac carrying *NusA* fused to N-terminus of *bLIS*	This work
pETDuet-*CmR29*bLIS*	pETDuet-tac carrying *CmR29* fused to N-terminus of *bLIS*	This work
pETDuet-*GST*bLIS*	pETDuet-tac carrying *GST* fused to N-terminus of *bLIS*	This work
pETDuet-*CmR29*bLIS* (max)	pETDuet-tac containing fused *CmR29*bLIS* with TIR of 176000	This work
pETDuet-*CmR29*bLIS* (max)-*AgGPPS*	pETDuet-*CmR29*bLIS* (max) carrying *A. grandis GPPS* at second multiple cloning site	This work
pETDuet-*CmR29*bLIS* (max)-*IspA*	pETDuet-*CmR29*bLIS* (max) carrying *E. coli IspA* at second multiple cloning site	This work
pETDuet-*CmR29*bLIS* (max)-*IspA(S80F)*	pETDuet-*CmR29*bLIS* (max) carrying mutated *E. coli IspA (S80F)* at second multiple cloning site	This work
pETDuet-*CmR29*bLIS* (max)-*Erg20*	pETDuet-*CmR29*bLIS* (max) carrying *S. cerevisiae Erg20* at second multiple cloning site	This work
pETDuet-*CmR29*bLIS* (max)-*Erg20 (F96W)*	pETDuet-*CmR29*bLIS* (max) carrying mutated *S. cerevisiae Erg20 (F96W)* at second multiple cloning site	This work
pETDuet-*CmR29*bLIS* (max)-*Erg20 (N127W)*	pETDuet-*CmR29*bLIS* (max) carrying mutated *S. cerevisiae Erg20 (N127W)* at second multiple cloning site	This work

pETDuet-*CmR29*bLIS* (max)-*Erg20 (F96W/N127W)*	pETDuet-*CmR29*bLIS* (max) carrying mutated *S. cerevisiae Erg20 (F96W/N127W)* at second multiple cloning site	This work
Strains		
BL21	F^- *dcm ompT hsdS* (rB^-mB^-) gal λ^s	Invitrogen
CIBTS1758	BL21, *glmS-pstS*:: P_L^* MK_{MM} PMK_{SC} PMD_{SC} idi_{SC}, Δ*idi*:: PGI^* idi_{SC}., P_L^{**} *dxs*, PGI^**dxr*	[S1]
WX3000	*E. coli* CIBTS1758 harboring pAGES and pHGFH	This work
WX4000	*E. coli* WX3000 harboring pETDuet-*laLIS*	This work
WX5000	*E. coli* WX3000 harboring pETDuet-*ofLIS*	This work
WX6000	*E. coli* WX3000 harboring pETDuet-*bLIS*	This work
WX6000MK	*E. coli* WX3000 harboring pETDuet-*bLIS-MK*	This work
WX6100	*E. coli* WX3000 harboring pETDuet-*bLIS* (0.1k)	This work
WX6200	*E. coli* WX3000 harboring pETDuet-*bLIS* (0.2k)	This work
WX6300	*E. coli* WX3000 harboring pETDuet-*bLIS* (0.5k)	This work
WX6400	*E. coli* WX3000 harboring pETDuet-*bLIS* (5k)	This work
WX6500	*E. coli* WX3000 harboring pETDuet-*bLIS* (50k)	This work
WX6600	*E. coli* WX3000 harboring pETDuet-*bLIS* (max)	This work
WX6010	*E. coli* WX3000 harboring pETDuet-*MBP*bLIS*	This work
WX6020	*E. coli* WX3000 harboring pETDuet-*NusA*bLIS*	This work
WX6030	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS*	This work
WX6040	*E. coli* WX3000 harboring pETDuet-*GST*bLIS*	This work
WX6630	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)	This work
WX6631	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*IspA*	This work
WX6632	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*IspA (S80F)*	This work
WX6633	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*Erg20*	This work
WX6634	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*Erg20 (F96W)*	This work
WX6635	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*Erg20 (N127W)*	This work
WX6636	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*Erg20 (F96W/N127W)*	This work
WX6637	*E. coli* WX3000 harboring pETDuet-*CmR29*bLIS* (max)-*AgGPPS*	This work

Table S3. Different TIRs strength and corresponding RBS sequences

TIRs	RBS sequences 5' → 3'
ori	AGGAAA
0.1k	CAGAATTTGTAGTAATATTCCCGCTA
0.2k	TTCGGCTACACGAGTAACTATACCAGG
0.5k	GACAACGAAGGCACCGCTCCA
5k	GATTATCCGCACGGGAGGTAGG
50k	GAGAAGTAAGGAGTAGTA
176k	TACAAGTACGGAAGTAAACGATAAGGAGGTTTTTT

Table S4. Sequences of fusion tags used in this study

Fusion tags	Sequences
MBP	MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQT
NusA	MNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEA
CmR29	MEKKITGYTTVDISQWHRKEHFEAFQSVA
GST	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK

Table S5. Genebank ID and sequences of LISs and GPPSs used in this study

Monoterpene synthases (Genebank number)	Sequences
ofLIS (ACM92062.1)	MGHHHHHHRRSANYRPSAWDDNYIQSLNTVQYGAKKHLTREAEL IEQVKMLLDPPLLTTEMEAVQQLDLIDVSQNLGLSYHFKDQIKALS FIYYDHWEKYDSESVVNDLYFTSLGFRLFRQHGFPVSQEVFDCFKN ENGACFESGTEDDTKGVLQLYEASYLVRPGEDTLEEARQFATKSLK RKLEKDGANIIAHSLEIPLHWRIQRLEARWFLDMYEKQDRHDMNQ VILELAKLDFNIVRATQQEELKDLSRWWVESGLPEKLPFARDRHVE SYFWAIALFEPHQYGYERKVAAKIITMATSLDDVYDVYGTLGELQL FTNFINRWDTKSIEQLPYYMKLYYFGLYNSVSELGYDTLKEKGFILP YLKRSWEDLIDSYLKEAQWINNGYTPSLEEYLNNACISFGATPVIM HVFFTLSVSIDKPVIECLYRTHNILRYVGMLVRLTDDLSTSSGEMER GDELKTIELYMKERGATEIEAQEHIRFLINKTWKKMNKEVAIADCPP FTLATNLGRMAHFMYVDGDGNGNRHSQIHQRIMSLLFTQYALI
laLIS (ABB73045.1)	MGRRSGNYRPSAWDSNYIQSLNSQYKEKKCLTRLEGLIEQVKELK GTKMEAVQQLELIDDSQNLGLSYYFQDKIKHILNLIYNDHKYFYDS EAEGMDLYFTALGFRLFRQHGFKVSQEVFDRFKNENGTYFKHDDT KGLLQLYEASFLVREGEETLEQAREFATKSLQRKLDEDGDGIDANI ESWIRHSLEIPLHWRAQRLEARWFLDAYARRPDMNPVIFELAKLN FNIVQATQQEELKALSRWWSSLGLAEKLPFVRDRLVESYFWAIPLF EPHQYGYQRKVATKIITLITSLDDVYDIYGTLDELQLFTNLFERWD NASIGRLPEYLQLFYFAIHNFVSEVAYDILKEKGFTSIVYLQRSWVD LLKGYLKEAKWYNSGYTPSLEEYFDNAFMTIGAPPVLSQAYFTLG SSMEKPIIESMYEYDNILRVSGMLVRLPDDLGTSSFEMERGDVPKS VQLYMKETNATEEEAVEHVRFLNREAWKKMNTAEAAGDSPLVS DVVAVAANLGRAAQFMYFDGDGNQSSLQQWIVSMLFEPYA
bLIS (D5SL78.1)	MQEFEFAVPAPSRVSPDLARARARHLDWVHAMDLVRGEEARRRYE FSCVADIGAYGYPHATGADLDLCVDVLGWTFLFDDQFDAGDGRER DALAVCAELTDLLWKGTAATAASPPIVVAFSDCWERMRAGMSDA WRRRTVHEWVDYLAGWPTKLADRAHGAVLDPAAHLRARHRTICC RPLFALAERVGGYEVPRRAWHSSRLDGMRFTTSDAVIGMNELHSFE KDRAQGHANLVLSLVHHGGLTGPEAVTRVCDLVQGSIESFLRLRSG LPELGRALGVEGAVLDRYADALSAFCRGYHDWGRGASRYTTRDH PGDLGLENLVARSSG
GPP synthases (Genebank number)	Sequences
AgGPPS (AAN01134.1)	MFDFNKYMDSKAMTVNEALNKAIPLRYPQKIYESMRYSLLAGGK RVRPVLCIAACELVGGTEELAIPTACAIEMIHTMSLMHDDLPCIDND DLRRGKPTNHKIFGEDTAVTAGNALHSYAFEHIAVSTSKTVGADRIL RMVSELGRATGSEGVMGGQMVDIASEGDPSIDLQTLEWIHIHKTA MLLECSVVCGAIIGGASEIVIERARRYARCVGLLFQVVDDILDVTKS SDELGKTAGKDLISDKATYPKLMGLEKAKEFSDELLNRAKGELSCF DPVKAAPLLGLADYVAFRQN

IspA (AAC73524.1)	MDFPQQLEACVKQANQALSRFIAPLPFQNTPVVETMQYGALLGGK RLRPFLVYATGHMFGVSTNTLDAPAAAVECIHAYSLIHDDLPAMD DDDLRRGLPTCHVKFGEANAILAGDALQTLAFSILSDADMPEVSD RDRISMISELASASGIAGMCGGQALDLDAEGKHVPLDALERIHRHK TGALIRAAVRLGALSAGDKGRRALPVLDKYAESIGLAFQVQDDIL DVVGDTATLGKRQGADQQLGKSTYPALLGLEQARKKARDLIDDA RQSLKQLAEQSLDTSALEALADYIIQRNK
Erg20 (DAA08636.1)	MASEKEIRRERFLNVFPKLVEELNASLLAYGMPKEACDWYAHSLN YNTPGGKLNRGLSVVDTYAILSNKTVEQLGQEEYEKVAILGWCIE LLQAYCLVADDMMDKSITRRGQPCWYKVPEVGEIAINDAFMLEA AIYKLLKSHFRNEKYYIDITELFHEVTFQTELGQLMDLITAPEDKVD LSKFSLKKHSFIVTFKTAYYSFYLPVALAMYVAGITDEKDLKQARD VLIPLGEYFQIQDDYLDCFGTPEQIGKIGTDIQDNKCSWVINKALEL ASAEQRKTLDENYGKKDSVAEAKCKKIFNDLKIEQLYHEYEESIAK DLKAKISQVDESRGFKADVLTAFLNKVYKRSK

Reference

S1. Yang C, Gao X, Jiang Y, Sun B, Gao F, Yang S: **Synergy between methylerythritol phosphate pathway and mevalonate pathway for isoprene production in Escherichia coli.** *Metabolic Engineering* 2016, **37:**79-91.