Additional file

Highly efficient biosynthesis of (Z)-γ-bisabolene with a new sesquiterpene synthase AcTPS5 by dual cytoplasmic-peroxisomal engineering in *Saccharomyces cerevisiae*

Jiajia Liu, Ge Yao, Xiukun Wan, Fuli Wang, Penggang Han, Shaoheng Bao, Kang Wang, Tianyu Song, Hui Jiang*

State Key Laboratory of NBC Protection for Civilian, Beijing, 102205, People's Republic of China

* Correspondence

Content

Table S1 Bisabolene synthases from different organisms.

ID No.	Protein	Accession No.	Organism	Reference
1	Ag1	AAC24192.1	*Abies grandis*	[46]
2	PmeTPS3	—	*Pseudotsuga menziesii*	[47]
3	ZoTps1	BAI67934.1	*Zingiber officinale*	[48]
4	GbTPS2	AIU94290.1	*Ginkgo biloba*	[49]
5	SaBS	AIV42941.1	*Santalum album*	[50]
6	AtTPS12	NP_193064.2	*Arabidopsis thaliana*	[51]
7	AtTPS13	AEE83260.1	*Arabidopsis thaliana*	[51]
8	HaTPS12-K7	AME16497.1	*Helianthus annuus*	[52]
9	HaTPS12-K11	AME16498.1	*Helianthus annuus*	[52]
10	NvIDS1	A0A386JV86.1	*Nezara viridula*	[53]
11	SaTPS2	AZM65215.1	*Santalum album*	[54]
12	SaTPS3	AZM65216.1	*Santalum album*	[54]
13	PvTPS03	—	*Panicum virgatum L.*	[55]
14	PvTPS17	—	*Panicum virgatum L.*	[55]
15	PvTPS83	—	*Panicum virgatum L.*	[55]
16	PvTPS20	—	*Panicum virgatum L.*	[55]
17	BbS	WP_035857999	*Cryptosporangium arvum*	[21]
18	PcSTS-08	BCX55502.1	*Phanerochaete chrysosporium*	[22]
19	CcTPS2	WGM49142.1	*Colquhounia coccinea*	[11]
20	Tps1A	—	*Antrodia cinnamomea*	[3]
37	Tps2A	—	*Antrodia cinnamomea*	[3]

Table S2 Strains and plasmids.

Strains or plasmids	Characteristics	Derived from	Source
	Strains		
JCR27	ChrXII-2Δ:: *HygR*-P_{TEF1}-*Cas9*; ChrXI-3Δ:: P_{GAL1}-*ERG8*, P_{GAL10}-*tHMG1*, P_{GAL7}-*ERG12*; ChrX-3Δ:: P_{GAL1}-*ERG13*, P_{GAL10}-*tHMG1*; ChrXII-4Δ:: P_{GAL1}-*IDI1*, P_{GAL10}-*ERG10*, P_{GAL7}-*MVD1*	[23]	[23]
LSc5	pGAL1-*Actps5*, free replicating plasmid	[20]	[20]
JCR29	*GAL1*Δ:: P_{GAL1}-*ERG10*, P_{GAL10}-*ERG13*; 911bΔ:: P_{GAL1}-*tHMG1*, P_{GAL10}-*ERG12*; *LPP1*Δ:: P_{GAL1}-*ERG8*, P_{GAL10}-*MVD1*; *LEU2*Δ:: P_{GAL1}-*IDI1*, P_{GAL10}-*ERG20*	JCR27	This study
LSc90	*URA3*Δ:: *Actps5*-ePTS1	JCR29	This study
LSc99	*URA3*Δ:: *Actps5*-ePTS1; 308aΔ:: P_{GAL1}-*tHMG1*-ePTS1, P_{GAL10}-*Actps5*-ePTS1	JCR29	This study
LSc100	*URA3*Δ:: *Actps5*-ePTS1; 308aΔ:: P_{GAL1}-*tHMG1*-ePTS1, P_{GAL10}-*Actps5*-ePTS1; *ypl062w*Δ:: P_{GAL1}-*tHMG1*-ePTS1, P_{GAL10}-*Actps5*-ePTS1	JCR29	This study
LSc101	*pex11*Δ:: P_{GAL1}-*pex11*	LSc99	This study
LSc102	*vps1*Δ:: P_{GAL1}-*vps11*	LSc99	This study
LSc103	*pex30*Δ, *pex31*Δ, *pex32*Δ	LSc99	This study
LSc104	*Atg36*Δ	LSc99	This study
LSc105	*pex11*Δ:: P_{GAL1}-*pex11*; *vps1*Δ:: P_{GAL1}-*vps11*	LSc99	This study
LSc106	*pex11*Δ:: P_{GAL1}-*pex11*; *pex30*Δ, *pex31*Δ, *pex32*Δ	LSc99	This study
LSc107	*pex11*Δ:: P_{GAL1}-*pex11*; *Atg36*Δ	LSc99	This study
LSc108	*vps1*Δ:: P_{GAL1}-*vps11*; *pex30*Δ, *pex31*Δ, *pex32*Δ	LSc99	This study
LSc109	*vps1*Δ:: P_{GAL1}-*vps11*; *Atg36*Δ	LSc99	This study
LSc110	*pex30*Δ, *pex31*Δ, *pex32*Δ; *Atg36*Δ	LSc99	This study
LSc111	*pex11*Δ:: P_{GAL1}-*pex11*; *vps1*Δ:: P_{GAL1}-*vps11*; *Atg36*Δ	LSc99	This study
LSc112	*pex11*Δ:: P_{GAL1}-*pex11*; *pex30*Δ, *pex31*Δ, *pex32*Δ; *Atg36*Δ	LSc99	This study
LSc113	*vps1*Δ:: P_{GAL1}-*vps11*; *pex30*Δ, *pex31*Δ, *pex32*Δ; *Atg36*Δ	LSc99	This study
LSc114	*pex11*Δ:: P_{GAL1}-*pex11*; *vps1*Δ:: P_{GAL1}-*vps11*; *pex30*Δ, *pex31*Δ, *pex32*Δ; *Atg36*Δ	LSc99	This study
LSc123	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*	LSc102,	This study
LSc115	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*	LSc104,	This study
LSc125	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*	LSc105,	This study
LSc128	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*	LSc107	This study

LSc130	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*	LSc109	This study
LSc124	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*; *exg1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*; *dpp1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*	LSc102	This study
LSc116	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*; *exg1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*; *dpp1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*	LSc104	This study
LSc127	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*; *exg1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*; *dpp1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*	LSc105	This study
LSc129	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*; *exg1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*; *dpp1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*	LSc107	This study
LSc131	*rox1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*; *exg1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*; *dpp1*Δ:: P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*	LSc109	This study
LSc133	*TRP1*Δ:: P_{GAL10}-*Actps5*; P_{GAL1}-*tHMG1*	LSc124	This study
LSc126	*TRP1*Δ:: P_{GAL10}-*Actps5*; P_{GAL1}-*tHMG1*	LSc116	This study
LSc135	*TRP1*Δ:: P_{GAL10}-*Actps5*; P_{GAL1}-*tHMG1*	LSc127	This study
LSc136	*TRP1*Δ:: P_{GAL10}-*Actps5*; P_{GAL1}-*tHMG1*	LSc129	This study
LSc137	*TRP1*Δ:: P_{GAL10}-*Actps5*; P_{GAL1}-*tHMG1*	LSc131	This study
LSc149	*ACO1*Δ	LSc137	This study
LSc150	*ADH1*Δ	LSc137	This study
LSc152	*MLS1*Δ	LSc137	This study
LSc153	1309aΔ:: P_{GAL10}-*ALD2*, P_{GAL1}-*ALD6*	LSc137	This study
LSc154	1414aΔ:: P_{GAL10}-*ACS1*, P_{GAL1}-*ACS2*	LSc137	This study
LSc155	1041aΔ:: P_{GAL10}-*YlACL1*, P_{GAL1}- *YlACL2*, P_{GAL7}- *CTP1*	LSc137	This study
LSc156	*CIT2*Δ	LSc137	This study
LSc172	*ACO1*Δ, *MLS1*Δ	LSc137	This study
LSc164	*ACO1*Δ, *ADH1*Δ, *MLS1*Δ	LSc137	This study
LSc165	1414aΔ:: P_{GAL10}-*ACS1*, P_{GAL1}-*ACS2*	LSc153	This study
LSc166	1041aΔ:: P_{GAL10}-*YlACL1*, P_{GAL1}- *YlACL2*, P_{GAL7}- *CTP1*	LSc165	This study
LSc173	*ACO1*Δ, *MLS1*Δ	LSc165	This study
LSc174	P_{HXT1}-*ERG9*	LSc166	This study
LSc175	*GAL80*Δ:: P_{TRP1}-*TRP1*, P_{HIS3}-*HIS3*, P_{URA3}-*URA3*, P_{LEU2}-*LEU2*	LSc174	This study
Escherichia coli DH5α	Used for routine cloning	Purchase	Gibco BRL
Plasmids			
pRS462	Routine cloning and expressing vector, 2 μ origin, Amp^R, *URA*		this lab
pEASY-Blunt	Routing cloning vector		TransGen
pCas	Expressing Cas9 and gRNA vector for genome editing		[56]

SgRNA	contain the sequences of tRNA and gRNA scaffold	[56]
KlURA3	contain the truncated URA3	[56]
pLJJ39	pRS426, P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, P_{GAL7}-*ERG20*, pRS462 *URA*, AmpR	This study
pLJJ28	pCas, gRNA_*rox1*, AmpR	[20]
pLJJ42	pRS426, P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, *URA*, AmpR	This study
pLJJ148	pRS426, P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, *URA*, AmpR	This study
pLJJ30	pCas, gRNA_*exg1*, gRNA_*dpp1*, AmpR	[20]
pLJJ150	pRS426, P_{GAL10}-*Actps5*, P_{GAL1}-*tHMG1*, *URA*, AmpR	This study
pLJJ151	pCas, gRNA_*trp1*, AmpR	This study
pLJJ105	pRS426, P_{GAL10}- *ERG10*-ePTS1, P_{GAL1}- *ERG13*-ePTS1, *URA*, AmpR	This study
pLJJ106	pRS426, P_{GAL10}- *ERG8*-ePTS1, P_{GAL1}- *ERG12*-ePTS1, *URA*, AmpR	This study
pLJJ107	pRS426, P_{GAL10}- *tHMG1*-ePTS1, P_{GAL1}- *IDI1*-ePTS1, *URA*, AmpR	This study
pLJJ115	pRS426, P_{GAL10}- *MVD1*-ePTS1, P_{GAL1}- *ERG20*-ePTS1, *URA*, AmpR	This study
pLJJ108	pCas, gRNA_911b, gRNA_*GAL1*, gRNA_*Lpp1*, gRNA_LEU2, AmpR	This study
pLJJ147	pRS426, P_{GAL10}- *Actps5*-ePTS1, *URA*, AmpR	This study
pLJJ149	pCas, gRNA_URA3, AmpR	This study
pLJJ161	pRS426, P_{GAL10}- *Actps5*-ePTS1, P_{GAL10}- *tHMG1*-ePTS1, *URA*, AmpR	This study
pLJJ163	pCas, gRNA_308a, AmpR	This study
pLJJ162	pRS426, P_{GAL10}- *Actps5*-ePTS1, P_{GAL10}- *tHMG1*-ePTS1, *URA*, AmpR	This study
pLJJ164	pCas, gRNA_ *ypl062w*, AmpR	This study
pLJJ123	pRS426, P_{GAL2}- *pex11*, *TRP1*, TRP, AmpR	This study
pLJJ125	Blunt, P_{GAL10}- *vps1*, URA, AmpR	This study
pLJJ126	pCas, gRNA_ 511b, AmpR	This study
pLJJ117	Blunt, LB- pex30- RB, URA, AmpR	This study
pLJJ118	Blunt, LB- pex31- RB, URA, AmpR	This study
pLJJ119	Blunt, LB- pex32- RB, URA, AmpR	This study
pLJJ120	pCas, gRNA_*pex30*, gRNA_*pex31*, gRNA_*pex32*, AmpR	This study
pLJJ190	Blunt, LB- *ACO1*- RB, URA, AmpR	This study
pLJJ191	pCas, gRNA_*ACO1*, AmpR	This study
pLJJ189	Blunt, LB- *ACO1*- RB, TRP, AmpR	This study
pLJJ192	Blunt, LB- *MLS1*- RB, URA, AmpR	This study
pLJJ193	pCas, gRNA_*MLS1*, AmpR	This study
pLJJ194	Blunt, LB- *CIT2* -RB, URA, AmpR	This study
pLJJ195	pCas, gRNA_*CIT2*, AmpR	This study
pLJJ34	pRS426, pHXT1- *ERG9*	[20]
pLJJ132	pRS426, *URA3*, *TRP1*, *HIS3*, *LEU2*, AmpR, URA3	This study

pLJJ134	pCas, gRNA_*GAL80*, AmpR	This study

Table S3 Primers.

Primer name	**Primer sequence 5'-3'**
pPent1-5-F	AGCTGGAGCTCTAGTAGTTTAAACAGTCATGTAGCCGCCTAGCGAGCCTGGGT
pPent1-5-R	TGGGACGCTCGAAGGCTTTAATTTGCGATGTGCTGTCTGAACAGAATAAATGCGTTCT
pLJJ39-1-F	GCAAATTAAAGCCTTCGAGCGTCCCA
pLJJ39-1-R	ACAGGCCCCTTTTCCTTTGTCG
pLJJ39-2-F	CGACAAAGGAAAAGGGGCCTGTTTACTCGAGAGCTCTACAAG
pLJJ39-2-R	AATTTTTGAAAATTCAATATAAATGGCTGCTGATCCAACTGTTACTT
pLJJ39-3-F	TTATATTGAATTTTCAAAAATTCTTA
pLJJ39-3-R	TATAGTTTTTTCTCCTTGACGT
pLJJ39-4-F	AACGTCAAGGAGAAAAAACTATAATGGCTTCAGAAAAAGAAATTA
pLJJ39-4-R	TTTCAAGAAGGATAGTAAGCTGGCAAACTATTTGCTTCTCTTGTAAACTTTGTTCAAG
pLJJ39-5-F	TTTGCCAGCTTACTATCCTTCTTGA
pLJJ39-5-R	TTTTGAGGGAATATTCAACTGTTT
pLJJ39-6-F	AAAACAGTTGAATATTCCCTCAAAAATGGTTTTAACCAATAAAACA
pLJJ39-6-R	AATCATAAATCATAAGAAATTCGCTTAGGATTTAATGCAGGTGACGG
pLJJ39-7-F	GCGAATTTCTTATGATTTATGATT
pLJJ39-7-R	CCGGTAGAGGTGTGGTCAATAAGAGC
pLJJ39-8-F	CTCTTATTGACCACACCTCTACCGGATCTTAATAGACGAATGGACCGCTCAA
pLJJ39-8-R	ATGATATCGACAAAGGAAAAGGGGCCTGTTCAACCTCTGACCAAGACGGTTTCTTC
pPent1-4-F	TAGCCTAGTTTCTCGTTTAAACAACAGTTGCGCAGCCTGAATGGCGAATGGCGCGACG
pPent1-8-R	CGTCGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGTTTAAACGAGAAACTAGGCTA
pPent2-2-F	CAAAAGCTGGAGCTCTAGTAGTTTAAACTAATAGTACGTAATGTAGGAAGCCTGCTTC
pPent2-2-R	TTTGGGACGCTCGAAGGCTTTAATTTGCTTGACACCACGGATTGGTTCTCCGAGGGAA
pLJJ42-1-F	TCGACAAAGGAAAAGGGGCCTGTTTAGGATTTAATGCAGGTGACGGACCC
pLJJ42-1-R	GTAAGAATTTTTGAAAATTCAATATAAATGGTTTTAACCAATAAAACAGTC

pLJJ42-2-F	ACGTCAAGGAGAAAAAACTATAATGGCTGCTGATCCAACTGTTACT
pLJJ42-2-R	TATCGATTTCAATTCAATTCAATTTACTCGAGAGCTCTACAAGTTTGC
pLJJ42-3-F	ATTGAATTGAATTGAAATCGATAGA
pLJJ42-3-R	AACGAACGCAGAATTTTCGAGTTATTAA
pPent2-4-F	GTTTAATAACTCGAAAATTCTGCGTTCGTTTGGTTGGTTACTTCTTG AACCATACATT
pPent2-4-R	CCATTCGCCATTCAGGCTGCGCAACTGTTGTTTAAACTTGTAACACC AGATAATCCAA
pLJJ148-1-F	CTGGAGCTCTAGTAGTTTAAACTTTTATTGTTTCCTGTTGTTTTTCT
pLJJ148-1-R	GGACGCTCGAAGGCTTTAATTTGCGAGGATCCCGGATGAGGAATTA CA
pLJJ148-2-F	AACTCGAAAATTCTGCGTTCGTTGTGACTGCTTCCTCCAGGGTGAC ATC
pLJJ148-2-R	GCAACTGTTGTTTAAACTTTGTCGATCGGTTGTATATTTT
pLJJ150-1-F	AACAAAAGCTGGAGCTCTAGTAGTTTAAACCTCTATTCTGAAAACG GAAG
pLJJ150-1-R	GACGCTCGAAGGCTTTAATTTGCCCAAAACATCCTCCTTAGGTTGAT TAC
pLJJ150-2-F	AATAACTCGAAAATTCTGCGTTCGTTGAAAATGTTGGTGATGCGCTT AGA
pLJJ150-2-R	TGCGCAACTGTTGTTTAAACGTTTAAACCTTTCAAGAATTCCACATG TTA
pLJJ151-1-F	GGTCTCGGATCGGACAGGTGAACTTTTGGATGTTTTAGAGCTAGAA ATAGCAAGTTA
pLJJ151-1-R	GGTCTCTTGCGCAAGCCCGGAATCGAACC
pLJJ151-2-F	GGTCTCACGCATTTTTTTGGATCCATCTAAAGTCATTTCA
pLJJ151-2-R	GGTCTCAAAACCTTTTTCGATGATGTAGTTTCTGG
pLJJ105-1-F	CTGGAGCTCTAGTAGTTTAAACGACTAAATCTCATTCAGAAGAAG
pLJJ105-1-R	TCGCTCTTATTGACCACACCTCTACCGGTTTTCCAGAAGGTAAAACA A
pLJJ105-2-R	TTGGGTAGAGGTAGAAGATCTAAATTGTGAGCGAATTTCTTATGATT TATGATTTT
pLJJ105-3-F	TCACAATTTAGATCTTCTACCTCTACCCAATATCTTTTCAATGACAAT AGAGGAAG
pLJJ105-3-R	AAAGTAAGAATTTTTGAAAATTCAATATAAATGTCTCAGAACGTTTA CATTGTATCG
pLJJ105-4-F	ACTTTAACGTCAAGGAGAAAAAACTATAATGAAACTCTCAACTAAA CTTTGTTGGT
pLJJ105-4-R	CAATTTAGATCTTCTACCTCTACCCAATTTTTTAACATCGTAAGATCT TCTAAAT
pLJJ105-5-F	TTGGGTAGAGGTAGAAGATCTAAATTGTAAACAGGCCCCTTTTCCTT TGTCGATAT
pLJJ105-6-F	GACGACTCGAAGGCTTTAATTTGCAGTATACTTCTTTTTTTTACTTTG TTC

pLJJ105-6-R	CAGGCTGCGCAACTGTTGTTTAAACTCCAAATCCACATTATTTGGCG CA
pLJJ106-1-F	CAAAAGCTGGAGCTCTAGTAGTTTAAACATCTGTTGCAAAAATAGG CCTG
pLJJ106-1-R	GCTCTTATTGACCACACCTCTACCGGTTATATATACATTTATATTTATG C
pLJJ106-2-F	TCACAATTTAGATCTTCTACCTCTACCCAATTTATCAAGATAAGTTTC CGGATC
pLJJ106-2-R	TAAGAATTTTTGAAAATTCAATATAAATGTCAGAGTTGAGAGCCTTC AGTG
pLJJ106-3-F	CTTTAACGTCAAGGAGAAAAAACTATAATGTCATTACCGTTCTTAAC TTCTGCACC
pLJJ106-3-R	CAATTTAGATCTTCTACCTCTACCCAATGAAGTCCATGGTAAATTCGT GTTT
pLJJ106-4-F	GGACGCTCGAAGGCTTTAATTTGCAGAAGTAAATGAAAAATGAAAT AGCA
pLJJ106-4-R	CATTCAGGCTGCGCAACTGTTGTTTAAACCCAACAATATGGGTACG AGAGA
pLJJ107-1-F	GAACAAAAGCTGGAGCTCTAGTAGTTTAAACCTAAGTCTTCTAAGC TCTTTTCATAGT
pLJJ107-1-R	AGGTCGCTCTTATTGACCACACCTCTACCGGTAACACTTACAGAGTC CTATCAGGA
pLJJ107-2-F	TGGGTCCGTCACCTGCATTAAATCCTTGGGTAGAGGTAGAAGATCTA AATTGTG
pLJJ107-2-R	AAGAATTTTTGAAAATTCAATATAAATGGTTTTAACCAATAAAACAG
pLJJ107-3-F	TTAACGTCAAGGAGAAAAAACTATAATGACTGCCGACAACAATAGT ATG
pLJJ107-3-R	CAATTTAGATCTTCTACCTCTACCCAATAGCATTCTATGAATTTGCCT GTCA
pLJJ107-4-F	GGACGCTCGAAGGCTTTAATTTGCCCTTGGTAGAATATGACGAGTTT C
pLJJ107-4-R	CCATTCAGGCTGCGCAACTGTTGTTTAAACGGAATAACAGAGTTAA TTTTG
pLJJ115-1-F	AAAAGCTGGAGCTCTAGTAGTTTAAACATTTCTCAACAAGTAATTG GTT
pLJJ115-1-R	GGTCGCTCTTATTGACCACACCTCTACCGGTTCCTTTCTCTTACCAA AGTAAATAC
pLJJ115-2-F	TTACAATTTAGATCTTCTACCTCTACCCAATTCCTTTGGTAGACCAGT CTTTGCG
pLJJ115-2-R	GAATTTTTGAAAATTCAATATAAATGACCGTTTACACAGCATCCGTT ACCG
pLJJ115-3-F	TTTAACGTCAAGGAGAAAAAACTATAATGGCTTCAGAAAAAGAAAT TAGG
pLJJ115-3-R	CAATTTAGATCTTCTACCTCTACCCAATTTGCTTCTCTTGTAAACTTT

	GTTCAAGAA
pLJJ115-4-F	GGGACGCTCGAAGGCTTTAATTTGCAACGAATTCCCTACATTGAAG GTTC
pLJJ115-4-R	GCTGCGCAACTGTTGTTTAAACTAGTACTGAAGAGGAGGTCGACTA
pLJJ108-1-F	GGTCTCGGATCGTAATATTGTCTTGTTTCCCGTTTTAGAGCTAGAAAT AGCA
pLJJ108-1-R	GGTCTCTTGCGCAAGCCCGGAATCGAACC
pLJJ108-2-F	GGTCTCACGCACCTTGCCAATGAGTTCTACAGTTTTAGAGCTAGAA ATAGCAAGTTAA
pLJJ108-2-R	GGTCTCTATCCAAAAAAATGCGCAAGCCCGGAATCGAAC
pLJJ108-3-F	GGTCTCAGGATCCATCTAAAGTCATTTC
pLJJ108-3-R	GGTCTCTTTGCGATCATTTATCTTTCACTGCGGAG
pLJJ108-4-F	GGTCTCAGCAACTTCTTGTATTGCGTGGTTTTAGAGCTAGAAATAGC AAGTTAAAATA
pLJJ108-4-R	GGTCTCTAATCTGCGCAAGCCCGGAATCGAACC
pLJJ108-5-F	GGTCTCGGATTCTTTGCACTTCTGGAAGTTTTAGAGCTAGAAATAGC
pLJJ108-5-R	GGTCTCAAAACAAAAAAATGCGCAAGCCCGGAATCGAACCG
pLJJ147-1-F	GCTGGAGCTCTAGTAGTTTAAACACGCAGATAATTCCAGGTATT
pLJJ147-1-R	TAAAACTGAAAGTTCCAAAGAGAACTTCGTTTCCTGCAGGTTTTT
pLJJ147-2-F	CGTCAAGGAGAAAAAACTATAATGGCTGCTGATCCAACTGTTACT
pLJJ147-2-R	TTACAATTTAGATCTTCTACCTCTACCCAACTCGAGAGCTCTACAAG TTTGCATCAAT
pLJJ147-3-F	TTGGGTAGAGGTAGAAGATCTAAATTGTAAATTGAATTGAATTGAAA TCGATAGATC
pLJJ147-3-R	AACGAACGCAGAATTTTCGAGTTATTAAA
pLJJ147-4-F	TAACTCGAAAATTCTGCGTTCGTTGATAATGCATGTATACTAAACTC AC
pLJJ147-4-R	AGGCTGCGCAACTGTTGTTTAAACCGTTTAAGGGCAAATGTACTCT CGC
pLJJ161-1-F	GCTGGAGCTCTAGTAGTTTAAACGTTATATTTCAGAAAAATTATTCA A
pLJJ161-1-R	ACGCTCGAAGGCTTTAATTTGCTTAGATAAAAAGAAAAAAATTCGA A
pLJJ161-2-F	GCAAATTAAAGCCTTCGAGCGT
pLJJ161-2-R	TTGGGTAGAGGTAGAAGATCTAAATTGTAAACAGGCCCCTTTTCCTT TGTCGAT
pLJJ161-3-F	ACAATTTAGATCTTCTACCTCTACCCAAGGATTTAATGCAGGTGACG GACCCATCTTT
pLJJ161-3-R	GTATTACTGAAAGTTCCAAAGAGAAATGGTTTTAACCAATAAAACA GTCA
pLJJ161-4-F	ACGTCAAGGAGAAAAAACTATAATGGCTGCTGATCCAACTGTTACT
pLJJ161-4-R	ACAATTTAGATCTTCTACCTCTACCCAACTCGAGAGCTCTACAAGTT TGCATCAATGGAA
pLJJ161-5-F	TTGGGTAGAGGTAGAAGATCTAAATTGTAAATTGAATTGAATTGAAA

	TCGATAGA
pLJJ161-5-R	AACGAACGCAGAATTTTCGAGTTATTAAAC
pLJJ161-6-F	GTTTAATAACTCGAAAATTCTGCGTTCGTTATTACTTGTCTTCTTTGCTACATATT
pLJJ161-6-R	CCATTCAGGCTGCGCAACTGTTGTTTAAACTAGAACGAGTACAACACCCGATCCTCT
pLJJ162-1-F	AAGCTGGAGCTCTAGTAGTTTAAACACCTAACAGTTACAAATTGC
pLJJ162-1-R	GACGCTCGAAGGCTTTAATTTGCGCCCTTACGTGAGGGGCAGTGTC
pLJJ162-2-F	ATAACTCGAAAATTCTGCGTTCGTTCACCGACCATGTGGGCAAATTCGT
pLJJ162-2-R	CAGGCTGCGCAACTGTTGTTTAAACTTCTTCAAGTTAGATTCAGAAGAG
pLJJ149-1-F	GGTCTCGGATCGCAGACATTACGAATGCACAGTTTTAGAGCTAGAAATAGCAAGTT
pLJJ149-1-R	GGTCTCTTGCGCAAGCCCGGAATCGAACC
pLJJ163-1-F	GGTCTCGGATCCACTTGTCAAACAGAATATAGTTTTAGAGCTAGAAATAGCA
pLJJ164-1-F	GGTCTCGGATCAAGCAACCAGCACGTCGCCGGTTTTAGAGCTAGAAATAGCA
pLJJ123-1-F	GCTGGAGCTCTAGTAGTTTAAACAGTTTGCCATCGTGCAAATATAAG
pLJJ123-1-R	TTTTGCTGCTTTTACCGTCGCGTTTCCTCACATATTTGTCTTCACCCT
pLJJ123-2-F	AAACGCGACGGTAAAAGCAGCAAAA
pLJJ123-2-R	TATGAAAGAATTATTTTTTTTATTA
pLJJ123-3-F	TAATAAAAAAAATAATTCCTTTCTCATAATGGTCTGTGATACACTGGTATATC
pLJJ123-3-R	ATCTATCGATTTCAATTCAATTCAATCTATGTAGCTTTCCACATGTCTT
pLJJ123-4-F	TAACTCGAAAATTCTGCGTTCGTTCTCTATTCTGAAAACGGAAGAGGAGTAG
pLJJ123-4-R	TGGTGTTTATGCAAAGAAACCACT
pLJJ123-5-F	AGTGGTTTCTTTGCATAAACACCAATATAATCACTAAATAAAGTAGATTTT
pLJJ123-5-R	CAGGCTGCGCAACTGTTGTTTAAACCTGTGAAAGGATCGGACCCATAA
pLJJ125-1-F	CTGGAATTGCCCTTGTTTAAACTTTGCACATAAAGGGTGCCTTCAAC
pLJJ125-1-R	GAAATCTGTACCAACCGTATAGGTGAAAGA
pLJJ125-2-F	TTCACCTATACGGTTGGTACAGATTTCTTATATTGAATTTTCAAAAATTCTT
pLJJ125-2-R	TAATAGTAGAAATTAAATGCTCATCCATTATAGTTTTTTCTCCTTGACGTTA
pLJJ125-3-F	ATAATGGATGAGCATTTAATTTCTACTATTAACA
pLJJ125-3-R	CTAAACAGAGGAGACGATTTGACTAGCGC
pLJJ125-4-F	ATAACTCGAAAATTCTGCGTTCGTTCATTACGTCGTTTGCTTCAATTTTCTTCG
pLJJ125-4-R	AGCTACCAAGGCTGTTTCTGAGCAAAGTTTAAAC

pLJJ126-1-F	GGTCTCGGATCCAGTGTATGCCAGTCAGCCAGTTTTAGAGCTAGAAATAGCA
pLJJ117-1-F	GTTTAAACCGTTGACCGTTAAAGTGTATTTATCTAAATCC
pLJJ117-1-R	TGAAAATCAGTTTTTACACTCCGGAGG
pLJJ117-2-F	GATCCTCCGGAGTGTAAAAACTGATTTTCATGCTCGCTCCCGTTTTTACCTTTAC
pLJJ117-2-R	GTTTAAACAACAAGGCCGGTAAGCCATTCAGACAA
pLJJ118-1-F	GTTTAAACTCCGGCTGCCAGAGCGAACAATATTAA
pLJJ118-1-R	ACTATCAAATAAAGGGAAACCAAGGCTTG
pLJJ118-2-F	CAAGCCTTGGTTTCCCTTTATTTGATAGTTGAGTGCATGCCCCATATGGACAACA
pLJJ118-2-R	GTTTAAACCATTCAAATACACTTTAAGGCCAGAGT
pLJJ119-1-F	GTTTAAACTGTGTGTATTCTTTTTGAGTGCCATCC
pLJJ119-1-R	GCAGTTTAGAGGCTTAAAGCAATACTATTCTTTCCTTGAAATTAGCAAAATGATGTCC
pLJJ119-2-F	GAATAGTATTGCTTTAAGCCTCTAAACTGC
pLJJ119-2-R	GTTTAAACGCAAAATACATTGCTAAATATGAGGAG
pLJJ120-1-F	GGTCTCGGATCTTGCAGAGACGCTGCAACCGGTTTTAGAGCTAGAAATAGCA
pLJJ120-1-R	GGTCTCTTGCGCAAGCCCGGAATCGAACC
pLJJ120-2-F	GGTCTCACGCACAGCGCCGCTGGCTTGGCATGTTTTAGAGCTAGAAATAGCA
pLJJ120-2-R	GGTCTCTATCCAAAAAAATGCGCAAGCCCGGAATCGAACCG
pLJJ120-3-F	GGTCTCAGGATCCATCTAAAGTCATTTCAAC
pLJJ120-3-R	GGTCTCTCCATGATCATTTATCTTTCACTGCGGAGAAG
pLJJ120-4-F	GGTCTCAATGGTTAGGCATTATAAGCGGTTTTAGAGCTAGAAATAGCA
pLJJ120-4-R	GGTCTCAAAACAAAAAAATGCGCAAGCCCGGAATCGAACCGG
pLJJ190-1-F	ATCGATTTTGGGTTTATTTTTGATATTGTC
pLJJ190-1-R	TAATGGTAGTATTTATTACCTTCGTTCTACTA
pLJJ190-2-F	TAGTAGAACGAAGGTAATAAATACTACCATTAATGGTTCAAATATGGTTCTGCCTT
pLJJ190-2-R	AAGCCAAATTACAAGTAAAGATTAGTAAAG
pLJJ191-1-F	GGTCTCGGATCCATGGTCAAGATATTCAGAGGTTTTAGAGCTAGAAATAGCA
pLJJ189-1-F	CAATACCAGTCCGGATCAACTGG
pLJJ189-1-R	GGCTTTTTGAGTTTCTGGAATAGACATTG
pLJJ189-2-F	CAATGTCTATTCCAGAAACTCAAAAAGCCAACGACATTACTATATATATAATAT
pLJJ189-2-R	CTGCTGAAGCTACCATGTCTACAGTTCCTGATGCGGTATTTTCTCCTTACGCA
pLJJ189-3-F	AACTGTAGACATGGTAGCTTCAGCAG
pLJJ189-3-R	TCAACTCCAGTCCATAAATGGGGTTAT
pLJJ192-1-F	CCGTGTAAAACCATGAGGCTTCTTC

pLJJ192-1-R	TCTTAATTCTTTTATGTGCTTTTACTACTTTG
pLJJ192-2-F	AAGTAGTAAAAGCACATAAAAGAATTAAGATTGTGTCCACTAAGGC GACGCC
pLJJ192-2-R	TTATACATTTCCTGACTGTTGTTAACAAT
pLJJ193-1-F	GGTCTCGGATCGGGGTTAATTAATAGGTCAAGTTTTAGAGCTAGAA ATAGCA
pLJJ194-1-F	TGGCAAGAAACCAAACTACGGCATA
pLJJ194-1-R	TCGCTAAATCTCTCTTTTAGAGTCTTTTC
pLJJ194-2-F	ACTCTAAAAGAGAGATTTAGCGATTGGTCAAAAACATTGAAAGCAA ACT
pLJJ194-2-R	TCCCAAAAGGCGGACCATTGAGC
pLJJ195-1-F	GGTCTCGGATCCATTCAAAAGGACCTGCCCAGTTTTAGAGCTAGAA ATAGCA
pLJJ198-1-F	AAAAGCTGGAGCTCTAGTAGTTTAAACAACTTATGCAGATGTGACC ATAACCCTGG
pLJJ198-1-R	GGACGCTCGAAGGCTTTAATTTGCGATCCTAAACTGCGTCATAGTAA GTT
pLJJ198-2-F	TATCGACAAAGGAAAAGGGGCCTGTTTAGTTGTCCAAAGAGAGATT TATGTGAAC
pLJJ198-2-R	AAGAATTTTTGAAAATTCAATATAAATGCCTACCTTGTATACTGATAT CGAA
pLJJ198-3-F	TAACGTCAAGGAGAAAAAACTATAATGACTAAGCTACACTTTGACA CTGCTGAAC
pLJJ198-3-R	CTATCGATTTCAATTCAATTCAATTTACAACTTAATTCTGACAGCTTT TACTT
P199-1-F	GGTCTCGGATCCCTGTGGTGACTACGTATCCGTTTTAGAGCTAGAAA TAGCA
pLJJ200-1-F	CAAAAGCTGGAGCTCTAGTAGTTTAAACGTATATTTTATTGAGTCCT TCTTACA
pLJJ200-1-R	TTTTGGGACGCTCGAAGGCTTTAATTTGCCGGATAATTTGAGCAATG ATAGT
pLJJ200-2-F	GATATCGACAAAGGAAAAGGGGCCTGTTTACAACTTGACCGAATCA ATTAGATGTCT
pLJJ200-2-R	TAAGAATTTTTGAAAATTCAATATAAATGTCGCCCTCTGCCGTACAA TC
pLJJ200-3-F	ACTTTAACGTCAAGGAGAAAAAACTATAATGACAATCAAGGAACAT AAAGTAGTTTAT
pLJJ200-3-R	ATCTATCGATTTCAATTCAATTCAATTTATTTCTTTTTTTGAGAGAAA
pLJJ200-4-F	ATAACTCGAAAATTCTGCGTTCGTTTTTTTAAGACGCATCTCCAAAA AAGAAAAAG
pLJJ200-4-R	CCATTCAGGCTGCGCAACTGTTGTTTAAACAATCACCTCTCTCGAA AGTCAAAGGT
pLJJ201-1-F	GGTCTCGGATCGCGCCACAGTTTCAAGGGTCGTTTTAGAGCTAGAA ATAGCA

pLJJ196-1-F	AGCTGGAGCTCTAGTAGTTTAAACATATTGACCAGTAGTCATATTACTGGCA
pLJJ196-1-R	TTGGGACGCTCGAAGGCTTTAATTTGCAGGATATTAATTTTAGGGTCTC
pLJJ196-2-F	TCGACAAAGGAAAAGGGGCCTGTCTATGATCGAGTCTTGGCCTTGGAAACG
pLJJ196-2-R	AGTAAGAATTTTTGAAAATTCAATATAAATGTCTGCCAACGAGAACATCTCC
pLJJ196-3-F	TAACGTCAAGGAGAAAAAACTATAATGTCAGCGAAATCCATTCACGAGGCCGA
pLJJ196-3-R	ATTTTCAAGAAGGATAGTAAGCTGGCAAATTAAACTCCGAGAGGAGTGGAAG
pLJJ196-4-F	AACAGTTGAATATTCCCTCAAAAATGTCCAGTAAAGCTACCAAAAGTG
pLJJ196-4-R	TAAAAATCATAAATCATAAGAAATTCGCTCAGGCTAGCATAACTAAGACCTT
pLJJ196-5-F	TTATTGACCACACCTCTACCGGTAGTAAATTATCACTGTTTTCATCTAGA
pLJJ196-5-R	CCATTCAGGCTGCGCAACTGTTGTTTAAACCAATACCAGAGATGACTGGCCAAC
pLJJ197-1-F	GGTCTCGGATCTTATGTGCGTATTGCTTTCAGTTTTAGAGCTAGAAATAGCA
pLJJ132-1-F	CAATTTGGCACCTGCATACCCCATT
pLJJ132-1-R	TATATATATAGTAATGTCGTTAGATCTCTTGTTGTAGTCCATGACGGGAGT
pLJJ132-2-F	ATCTAACGACATTACTATATATATA
pLJJ132-2-R	CATAAAAAAATATAGAGTGTACTAGCCTGATGCGGTATTTTCTCCTTACG
pLJJ132-3-F	CGTAAGGAGAAAATACCGCATCAGGCTAGTACACTCTATATTTTTTTATG
pLJJ132-3-R	GAACATTAGGCACGGTTGAGACCGAAGATCTATCTGTGCGGTATTTCACACCGCATAG
pLJJ132-4-F	TCTTCGGTCTCAACCGTGCCTAATGTTC
pLJJ132-4-R	CCTGATGCGGTATTTTCTCCTTAC
pLJJ132-5-F	GTAAGGAGAAAATACCGCATCAGGAACTGTGGGAATACTCAGGTATCGTAA
pLJJ132-5-R	ATTAGGAATCATAGTTTCATGATT
pLJJ132-6-F	AATCATGAAACTATGATTCCTAATCCCAGTGCAGCGAACGTTATAAAAAC
pLJJ132-6-R	GGCCAGTGAATTCGAGCTCGGTACCCGGGAAAATATGACCCCCAATATGAGAAATTAA
pLJJ134-1-F	GGTCTCGGATCTCAAGCCGAATCCATTTATAGTTTTAGAGCTAGAAATAGCAAGTTAA

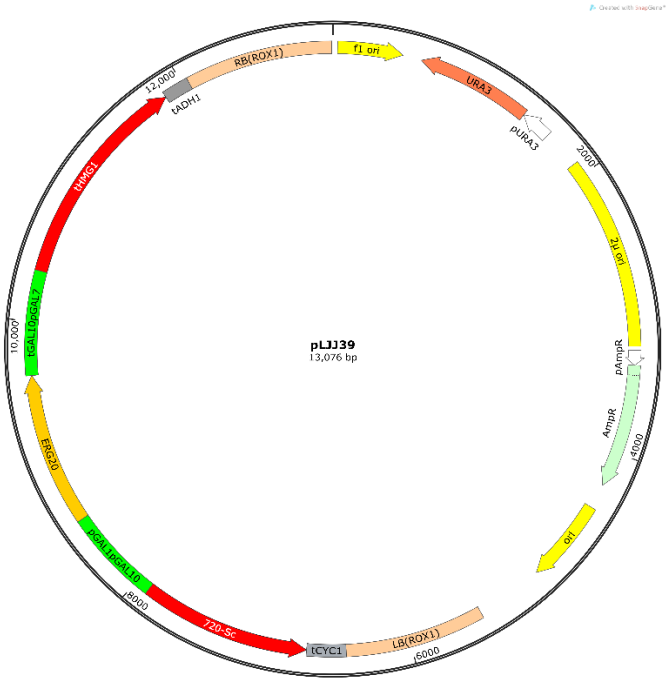

Fig. S1 Plasmid map for expressing M1 module

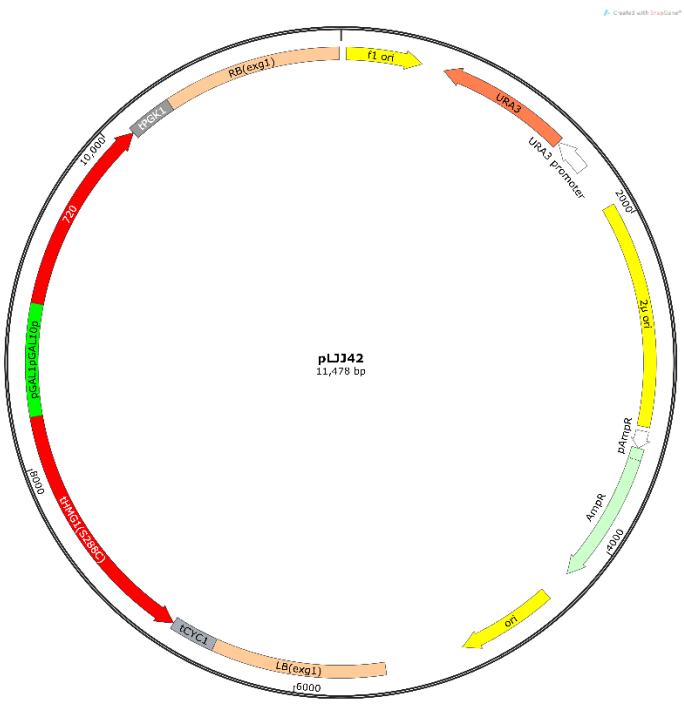

Fig. S2 Plasmid map for expressing M2 module

Fig. S3 Plasmid map for expressing M3 module

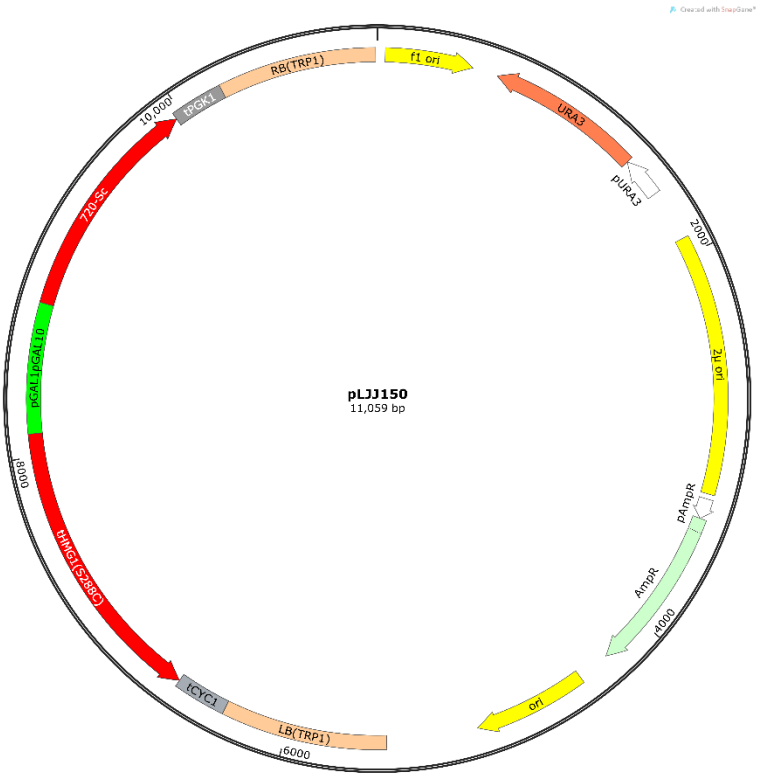

Fig. S4 Plasmid map for expressing M4 module

Fig. S5 Plasmid map for expressing M5 module

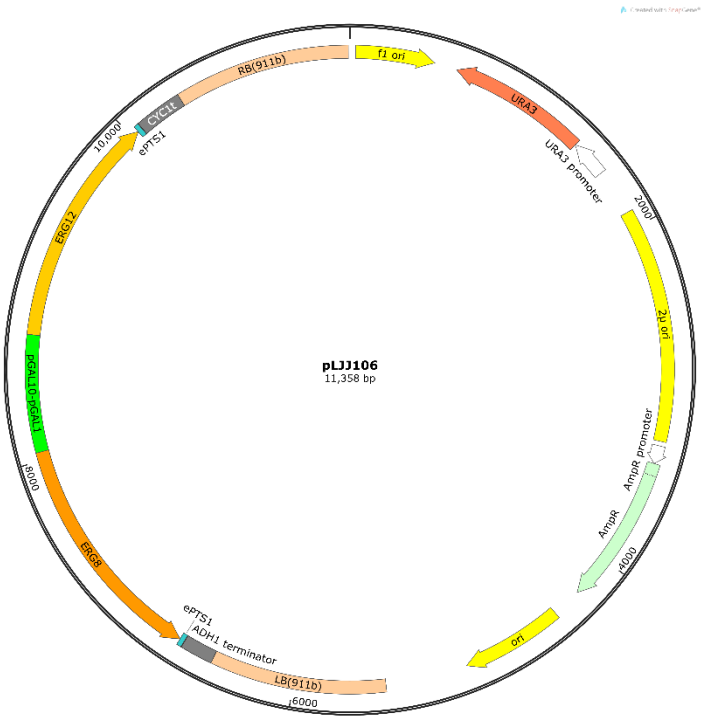

Fig. S6 Plasmid map for expressing M6 module

Fig. S7 Plasmid map for expressing M7 module

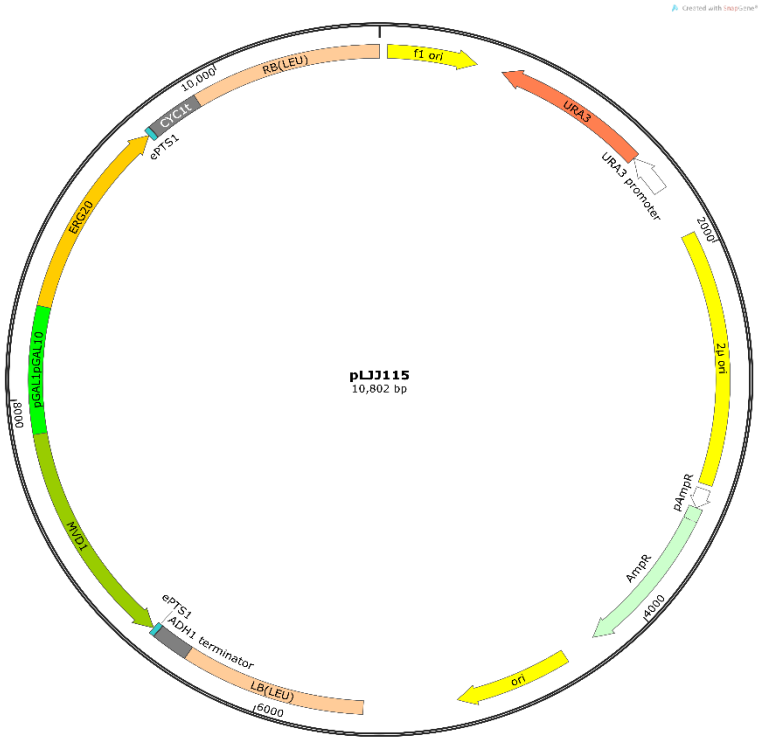

Fig. S8 Plasmid map for expressing M8 module

Fig. S9 Plasmid map for expressing M9 module

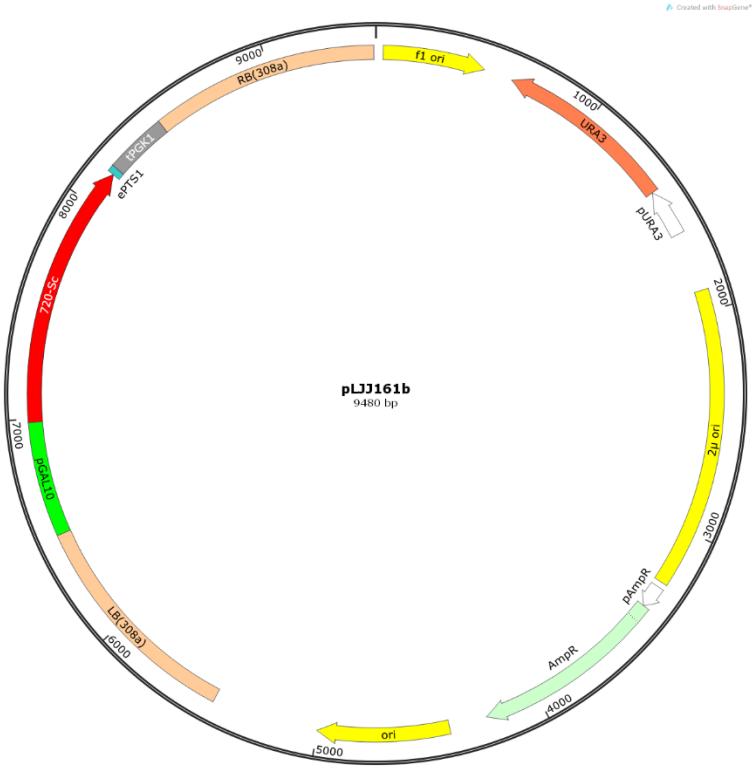

Fig. S10 Plasmid map for expressing M10 module

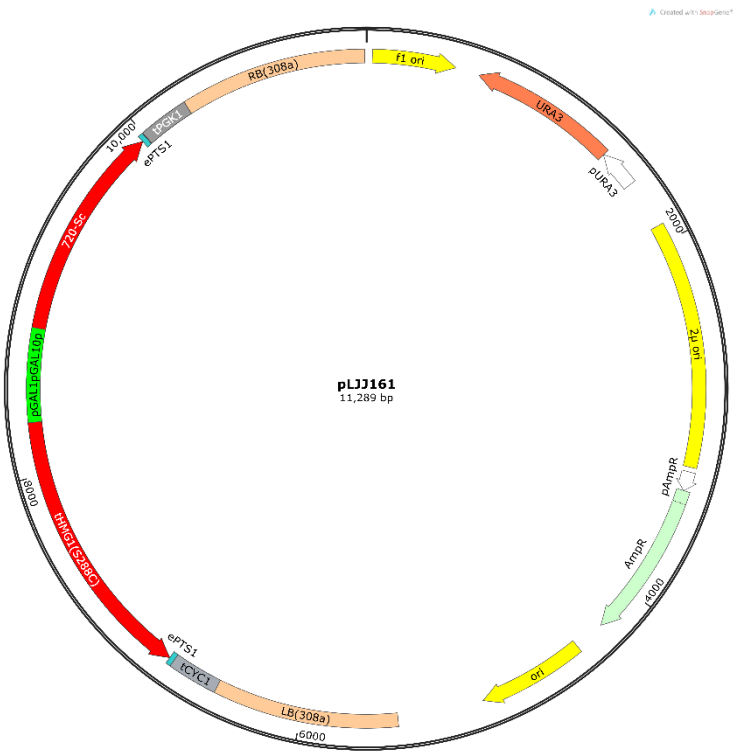

Fig. S11 Plasmid map for expressing M11 module

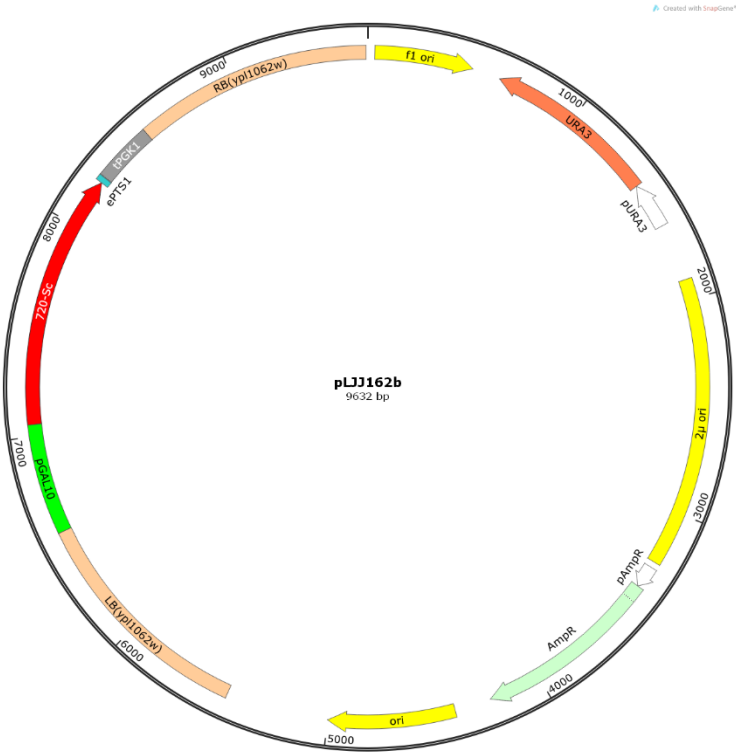

Fig. S12 Plasmid map for expressing M12 module

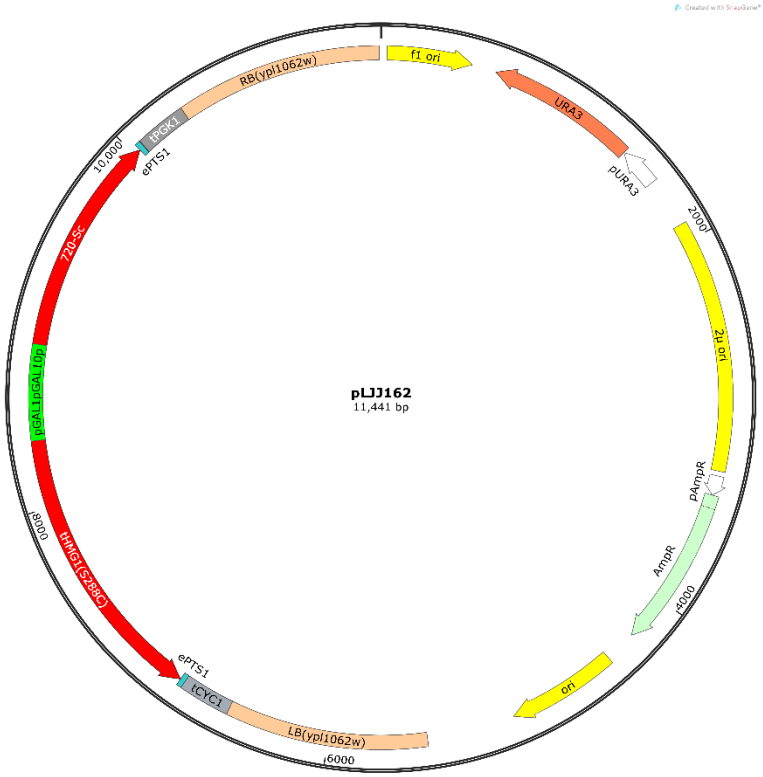

Fig. S13 Plasmid map for expressing M13 module

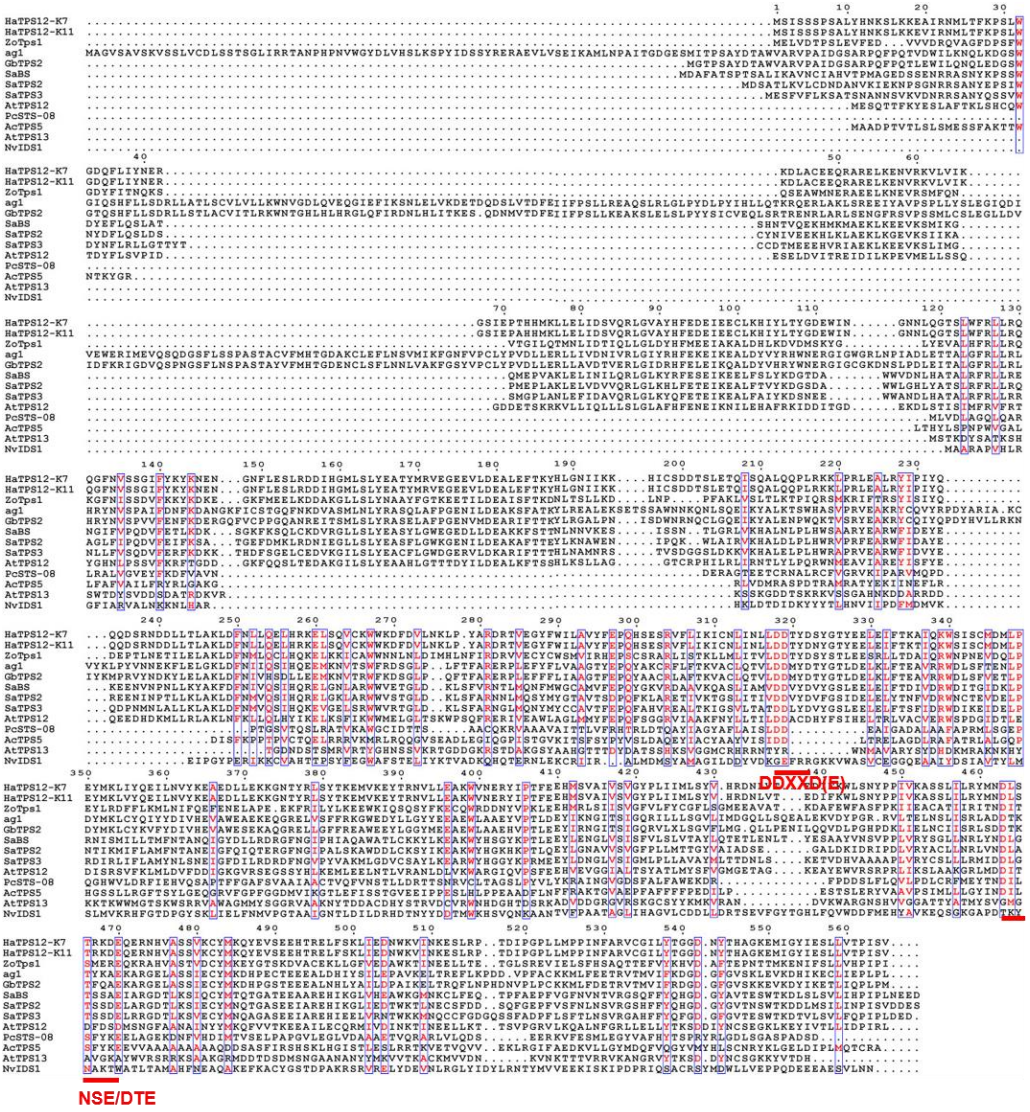

Fig. S14 Amino acids alignment of AcPS5 reported bisabolene synthases. The conserved motifs are marked respectively.

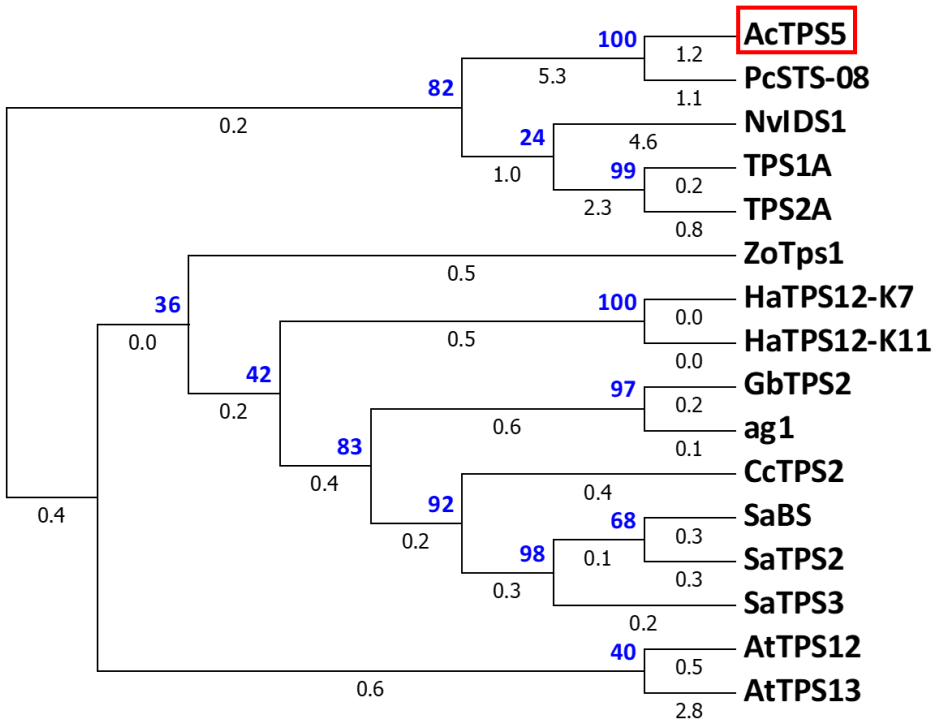

Fig. S15 Phylogenetic analysis of AcTPS5 and bisabolene synthases from other species. The proteins used were shown in Additional file 1: Table. S1. The branch lengths and bootstrap values are presented at the nodes in black and blue, respectively.

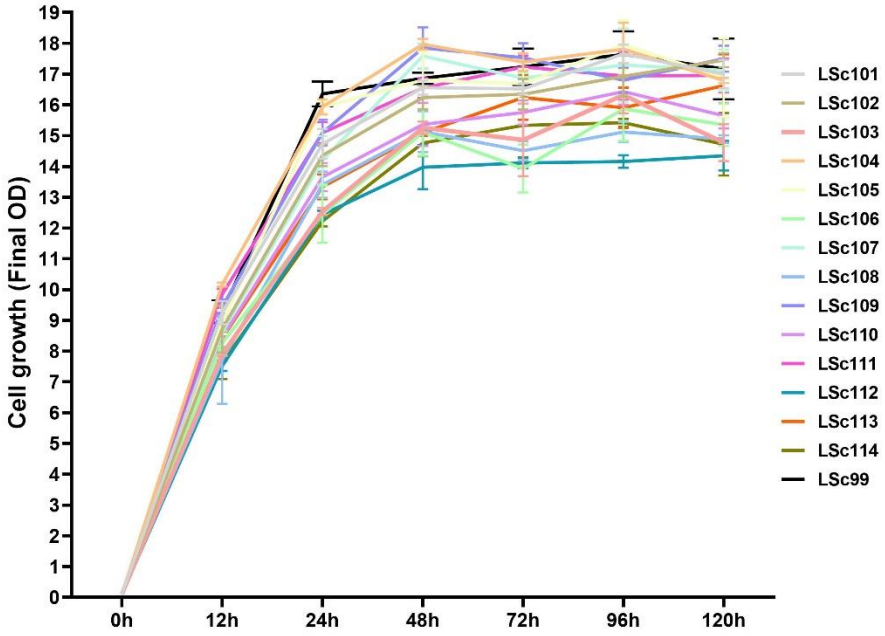

Fig. S16 Time course profiles of Cell growth. Error bars represent standard deviations from three independent experiments.