

Supporting information

Reprogramming the metabolism of oleaginous yeast for sustainably biosynthesizing the anticarcinogen precursor germacrene A

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Supplementary Fig. 1-7

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Supplementary References

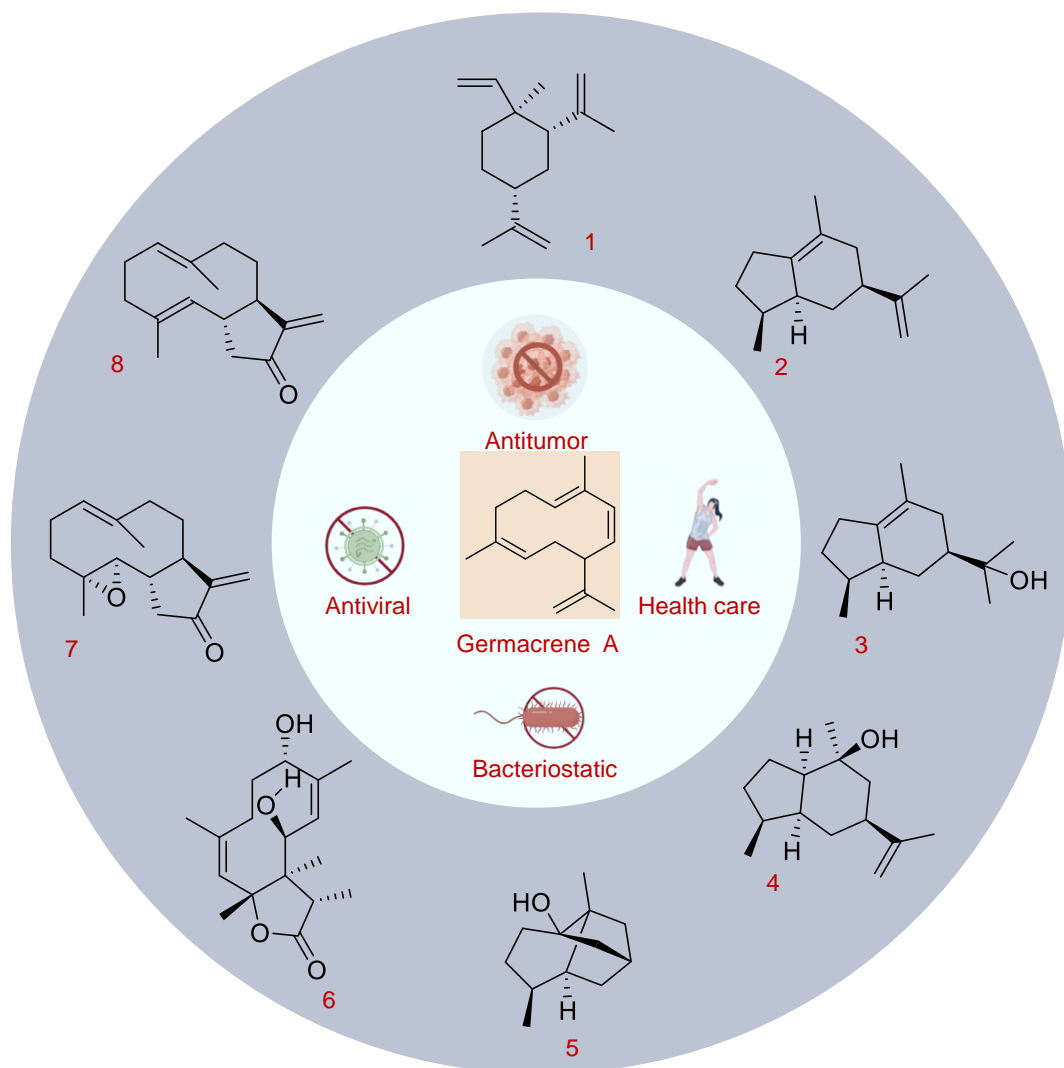


Fig. 1 The intermediate germacrene A can be converted into various products and their bioactivity. The sesquiterpenes and sesquiterpene lactone derived from germacrene A are :1, β -elemene; 2, δ -guaiene; 3, bulnesol; 4, pogostol; 5, patchoulol; 6, germacranolide; 7, parthenolide; 8, costunolide. Fig. S1 was created by Figdraw.

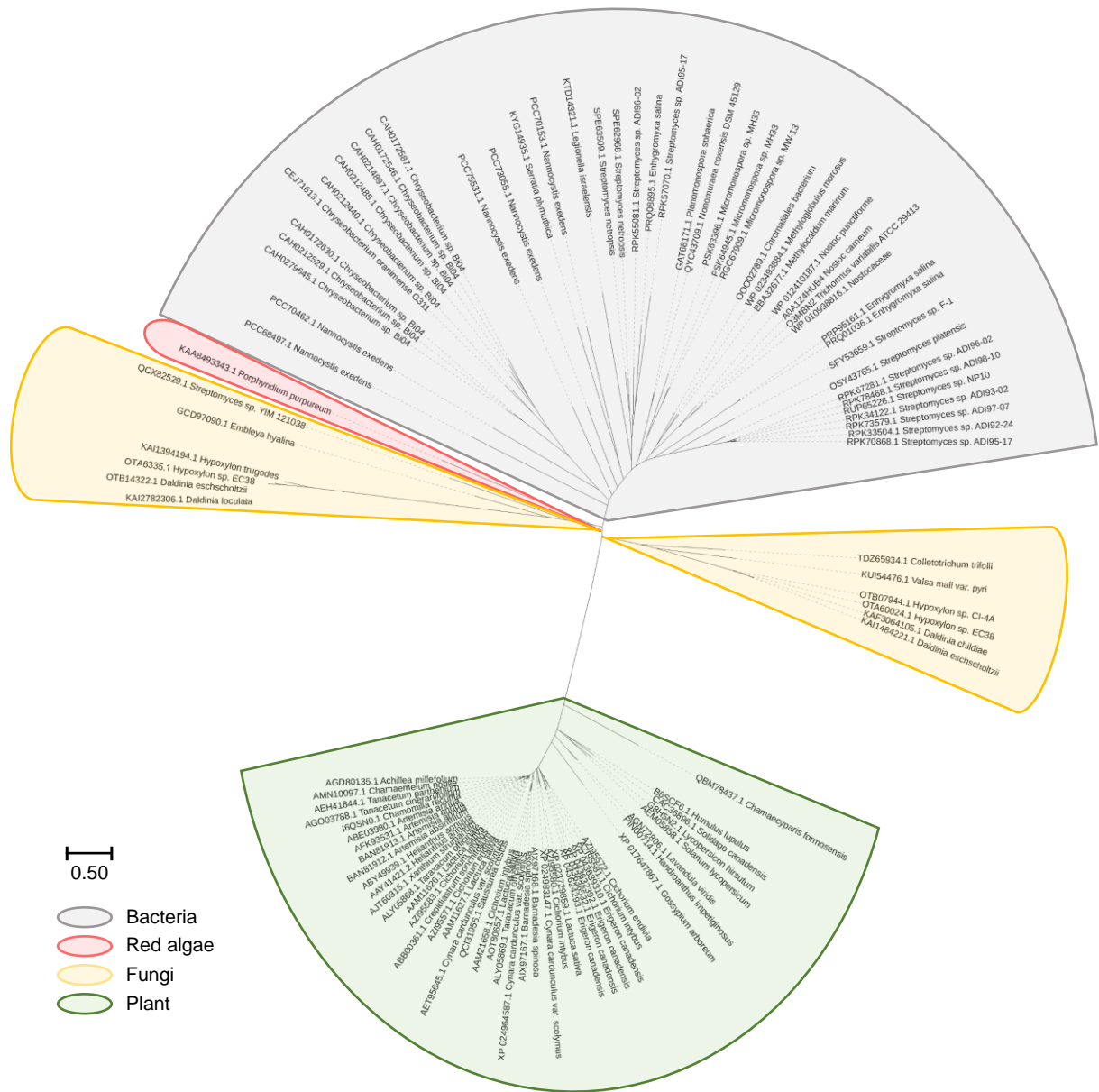


Fig. 2 Construction of a phylogenetic tree, exhibiting the evolution of germacrene A synthases. The enzyme sequences were download from NCBI or Uniport. The germacrene A synthases originating from bacteria, red algae, fungi and plant are shown in grayish, pink, light blue and reseda, respectively.

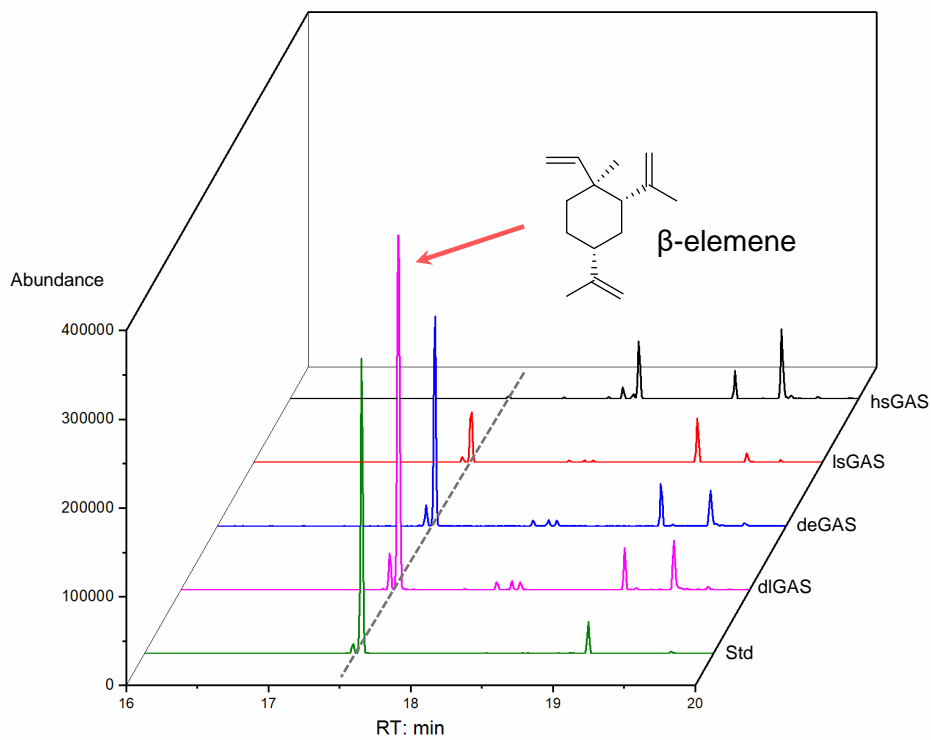
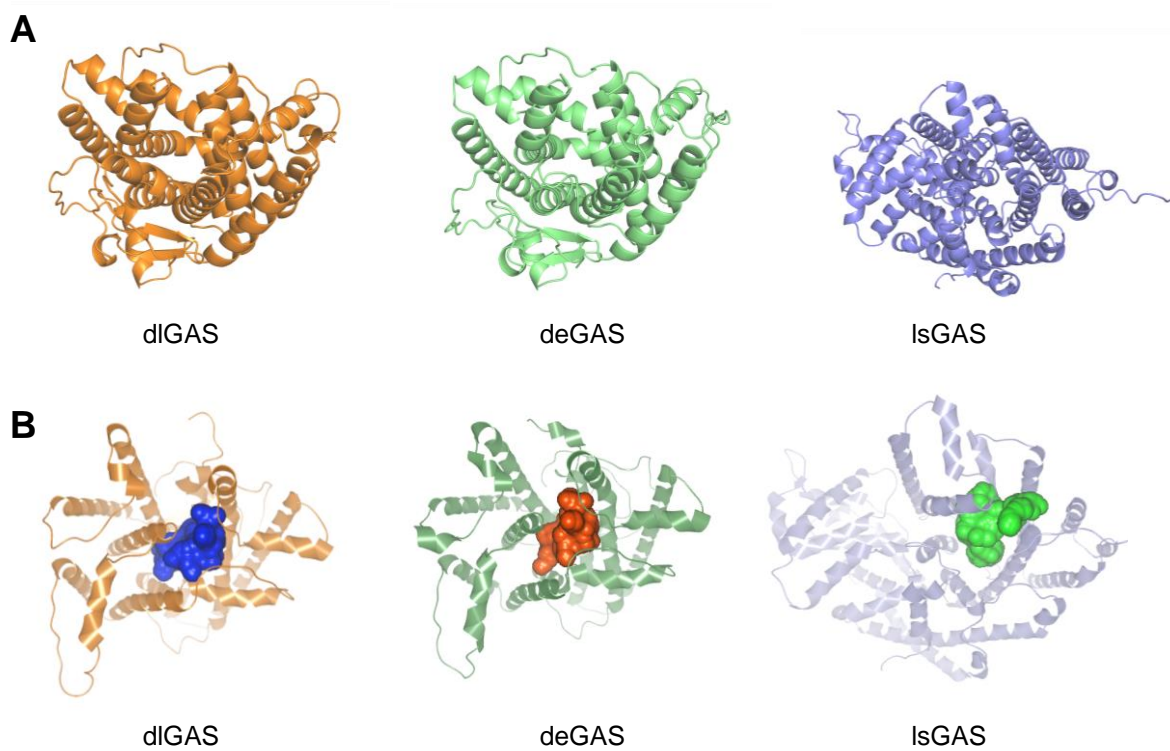


Fig. 3 GC-MS analysis of shake flask fermentation products in strains expressing dlGAS, deGAS, lsGAS and hsGAS. GC-MS profiles show the same retention time (17.5 minutes) as the β -elemene standard.



Structure ID	Pocket Volume (Å ³)
dlGAS	624.9
deGS	757.1
lsGAS	1617.8

Fig. 4 Computational analysis of the active cavities of dlGAS, deGAS and lsGAS.

(A) Protein models of GASs predicted using Alphafold2¹. (B) The volume of cavities calculated by CAVER Analyst 2.0².

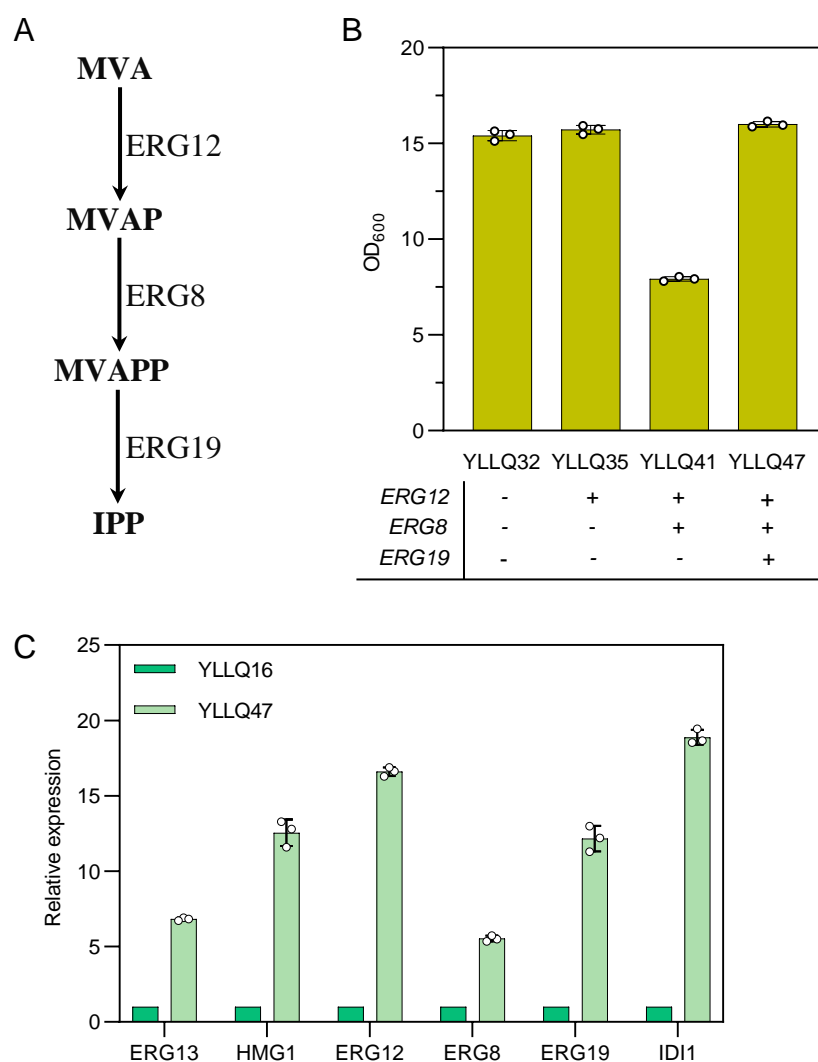


Fig. 5 Engineering the downstream mevalonate pathway to improve germacrene A production. (A) Schematic illustration of the enhanced conversion of mevalonate to IPP. (B) Effects of increasing downstream mevalonate pathway on the biomass of engineered strains. MVA, mevalonate; MVAP, 5-phosphomevalonate; MVAPP, 5-diphosphomevalonate; IPP, isopentenyl diphosphate. (C) qRT-PCR analysis of MVA pathway gene transcription levels of engineered strains YLLQ16 and YLLQ47. Error bars correspond to the SD of the mean ($n = 3$, corresponding to triplicate biological replicates).

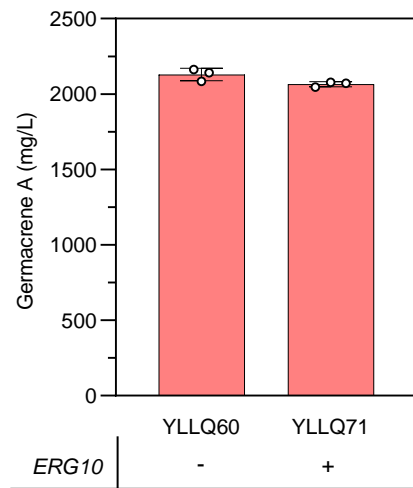


Fig. 6 Germacrene A production for the ERG10-engineered strain (YLLQ71) based on strain YLLQ60. Error bars correspond to the SD of the mean (n = 3, corresponding to triplicate biological replicates).

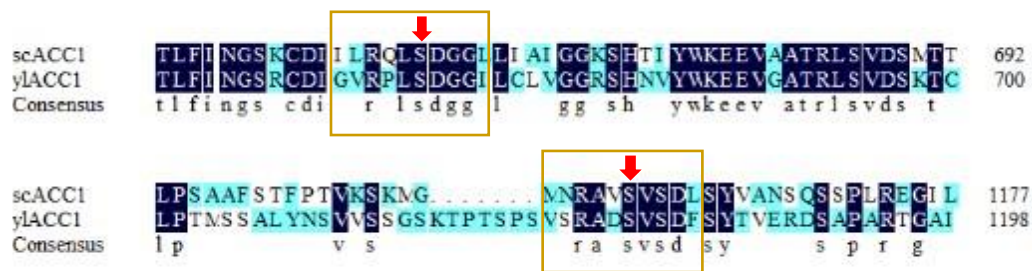


Fig. 7 Alignment of ACC1p amino acid sequences from *Y. lipolytica* and *S. cerevisiae*. BLAST analysis of the amino acid sequences was performed with ClustalW and the final format was generated with DNAMAN. Homologous residues are highlighted in deep blue, the brown frames represent the mutated sites and the red arrows represent the phosphorylation sites^{3,4}.

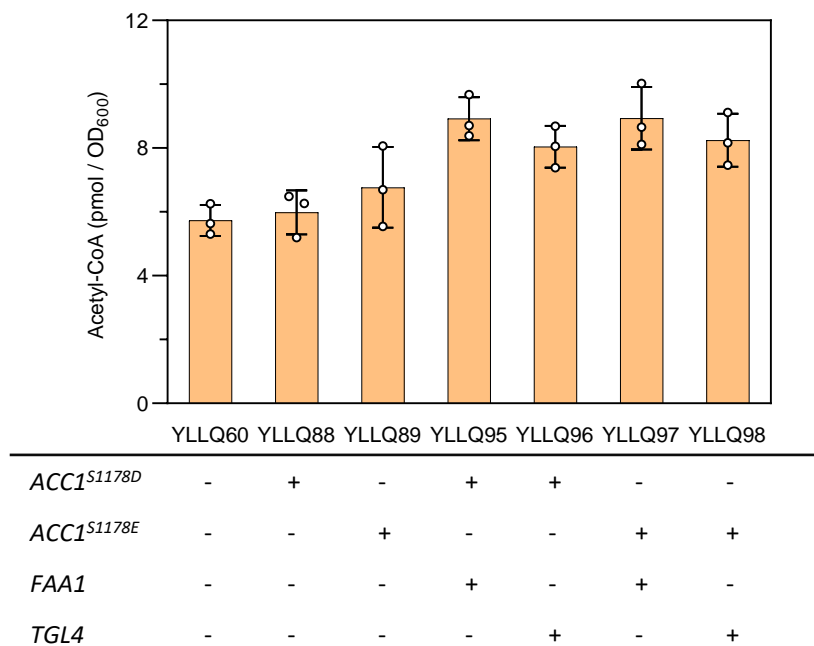


Fig. 8 Quantification of acetyl-CoA levels in engineered strains cultured for 24 h.

Error bars correspond to the SD of the mean (n = 3, corresponding to triplicate biological replicates).

Supplementary Tables

Table 1. Plasmids and gRNAs used in this study.

Name	Description	Resource
Plasmids		
pCfB3405	CEN1, AmpR, P _{EXP1} -Nat-T _{cyt}	5
gRNAs		
Int_C2	gctcatcggaagaactcct	5
Int_C3	aatagtctatggcttggg	5
Int_D1	gcagacgcagacggagactg	5
Int_E1	acaagcatacagccctcggg	5
Int_E3	cctgaaatcagtcgatggc	5
<i>P_{HMG1}</i>	acgaaggccacgaaatcaag	This study
<i>P_{IDI1}</i>	agatggagaaatgataccaa	This study
<i>P_{ERG13}</i>	tgcacgggtgtgacgtcggag	This study
<i>P_{ERG12}</i>	taaagttggaagaggacagg	This study
<i>P_{ERG8}</i>	tataggagatttacttatg	This study
<i>P_{ERG19}</i>	tggtgtgattagtgtttggg	This study
<i>P_{ERG9}</i>	actaacaagtacagttggga	This study
<i>P_{ERG10}</i>	tgtgacgtgagtgagagaga	This study
<i>ACCI^{S667}</i>	ggagttagaccttttctga	This study
<i>ACCI^{S1178}</i>	ggtgtacgaaaagtcggaga	This study

Table 2. Engineered strains used in this study.

Strains	Genotype or relevant characteristics	Resource
W29		NRRL
ZG03	W29 $\Delta ku70::Cas9, \Delta URA3$	This study
YLLQ01	ZG03 <i>Int_C2::P_{TEFin}-neGAS-T_{lip2}</i>	This study
YLLQ02	ZG03 <i>Int_C2::P_{TEFin}-spGAS-T_{lip2}</i>	This study
YLLQ03	ZG03 <i>Int_C2::P_{TEFin}-ppGAS-T_{lip2}</i>	This study
YLLQ04	ZG03 <i>Int_C2::P_{TEFin}-hsGAS-T_{lip2}</i>	This study
YLLQ05	ZG03 <i>Int_C2::P_{TEFin}-ctGAS-T_{lip2}</i>	This study
YLLQ06	ZG03 <i>Int_C2::P_{TEFin}-dlGAS-T_{lip2}</i>	This study
YLLQ07	ZG03 <i>Int_C2::P_{TEFin}-deGAS-T_{lip2}</i>	This study
YLLQ08	ZG03 <i>Int_C2::P_{TEFin}-hlGAS-T_{lip2}</i>	This study
YLLQ09	ZG03 <i>Int_C2::P_{TEFin}-lsGAS-T_{lip2}</i>	This study
YLLQ16	ZG03 <i>Int_C2::P_{TEFin}-dlGAS-GGGGS-ERG20-T_{lip2}</i>	This study
YLLQ17	ZG03 <i>Int_C2::P_{TEFin}-ERG20-GGGGS-dlGAS-T_{lip2}</i>	This study
YLLQ18	ZG03 <i>Int_C2::P_{TEFin}-dlGAS-T_{lip2}, P_{TEFin}-ERG20-T_{lip2}</i>	This study
YLLQ22	YLLQ16 <i>HMG1(-215, 3)::P_{TEFin}</i>	This study
YLLQ26	YLLQ22 <i>ID11(-216, 3)::P_{TEFin}</i>	This study
YLLQ32	YLLQ26 <i>ERG13(-194, 3)::P_{TEFin}</i>	This study
YLLQ35	YLLQ32 <i>ERG12(-84, 3)::P_{TEFin}</i>	This study
YLLQ41	YLLQ35 <i>ERG8(-61, 3)::P_{GPD}</i>	This study
YLLQ47	YLLQ41 <i>ERG19(-52, 0)::P_{GPD}</i>	This study
YLLQ52	YLLQ47 <i>ERG9(-499, 0)::P_{ERG9_50bp}</i>	This study
YLLQ58	YLLQ52 <i>Int_C3::P_{TEFin}-dlGAS-T_{pex20}</i>	This study
YLLQ59	YLLQ58 <i>Int_D1::P_{TEFin}-dlGAS-T_{cyc1}</i>	This study
YLLQ60	YLLQ59 <i>Int_E1::P_{TEFin}-dlGAS-T_{cyc1}</i>	This study
YLLQ61	YLLQ60 <i>Int_E3::P_{TEFin}-dlGAS-T_{lip2}</i>	This study
YLLQ71	YLLQ60 <i>ERG10(-87, 3)::P_{TEFin}</i>	This study
YLLQ86	YLLQ60 <i>ACC1^{S667D}</i>	This study
YLLQ87	YLLQ60 <i>ACC1^{S667E}</i>	This study
YLLQ88	YLLQ60 <i>ACC1^{S1178D}</i>	This study
YLLQ89	YLLQ60 <i>ACC1^{S1178E}</i>	This study
YLLQ90	YLLQ60 <i>Int_E3::P_{EXP1}-MFE1-T_{pex20}</i>	This study
YLLQ91	YLLQ60 <i>Int_E3::P_{EXP1}-FAA1-T_{pex20}</i>	This study
YLLQ92	YLLQ60 <i>Int_E3::P_{EXP1}-PEX10-T_{pex20}</i>	This study
YLLQ93	YLLQ60 <i>Int_E3::P_{EXP1}-POT1-T_{pex20}</i>	This study
YLLQ94	YLLQ60 <i>Int_E3::P_{EXP1}-TGL4-T_{pex20}</i>	This study
YLLQ95	YLLQ88 <i>Int_E3::P_{EXP1}-FAA1-T_{pex20}</i>	This study
YLLQ96	YLLQ88 <i>Int_E3::P_{EXP1}-TGL4-T_{pex20}</i>	This study
YLLQ97	YLLQ89 <i>Int_E3::P_{EXP1}-FAA1-T_{pex20}</i>	This study
YLLQ98	YLLQ89 <i>Int_E3::P_{EXP1}-TGL4-T_{pex20}</i>	This study

Table 3. List of primers and PCR fragments used in this work.

No.	Overlap PCR fragment	PCR fragment	Primer name	Sequence (5'-3')	
For evaluating the efficiency of GASs					
1	Int_C2-P _{TEF_{in}} - lsGAS-T _{lip2}	lsGAS	Int_C2-up-F	aattcgacgacctgaacct	
			Int_C2-up ^a	ccgccaaccgggtctcaagcgttgacggtt	
			Int_C2-up-R	ccatctaagacctacatt	
			Int_C2-dw ^a	tctgagtggcctatacctccgagtgtgcagc	
			Int_C2-dw-R	gcctgataaaaacgcctgtc	
			P _{TEF_{in}} ^a	P _{TEF_{in}} -F ^b	gagaccgggttggcggcgta
				P _{TEF_{in}} -R	ctgcggttagtactgcaaaaagtgc
				T _{lip2} -F ^b	cttctgttcggaatcaacctc
			T _{lip2} ^a	T _{lip2} -R	aggatagggccactcagatgcattcttgggc
					ggtc
					ttgcagtactaaccgcaggcccgctggac
					accaacgc
					gttgattccgaacagaagttacatagacaca
					gagccca
2	Int_C2-P _{TEF_{in}} - hlGAS-T _{lip2}	hlGAS	hlGAS-F	ttgcagtactaaccgcagctactcagatctt	
			hlGAS-R	cgctc	
3	Int_C2-P _{TEF_{in}} - deGAS-T _{lip2}	deGAS	deGAS-F	ttgcagtactaaccgcagcgacctacacct	
			deGAS-R	gttctttc	
4	Int_C2-P _{TEF_{in}} - dlGAS-T _{lip2}	dlGAS	dlGAS-F	gttgattccgaacagaagttagatgccttca	
			dlGAS-R	gagagg	
5	Int_C2-P _{TEF_{in}} - ctGAS-T _{lip2}	ctGAS	ctGAS-F	ttgcagtactaaccgcagggtctcagatcc	
			ctGAS-R	tggagcc	
6	Int_C2-P _{TEF_{in}} - hsGAS-T _{lip2}	hsGAS	hsGAS-F	gttgattccgaacagaagttacagcaccata	
			hsGAS-R	gacagag	
7	Int_C2-P _{TEF_{in}} - ppGAS-T _{lip2}	ppGAS	ppGAS-F	ttgcagtactaaccgcagatgcaaccacc	
			ppGAS-R	ctgctgcg	
				gttgattccgaacagaagttacagcagata	
				gacagagagc	
				ttgcagtactaaccgcagggtgcaagtggcc	
				gaggccgt	

			ppGAS-R	gttgattccgaacagaagttactccttagagtt gtatcgggc
8	Int_C2-P _{TEF_{in}} - spGAS-T _{lip2}	spGAS	spGAS-F	ttgcagtactaaccgcagcccacatcttcc acccectt
			spGAS-R	gttgattccgaacagaagttactgatccacct tcatcagag
9	Int_C2-P _{TEF_{in}} - neGAS-T _{lip2}	neGAS	neGAS-F	ttgcagtactaaccgcagaccgcagaatc gccgcctc
			neGAS-R	gttgattccgaacagaagttatcgacggcg ccgccgt
For fusing dlGAS and ERG20				
		dlGAS- GGGGS	dlGAS-GGGGS-F	gcagtactaaccgcagcaccatcacctg ttcttc
10	Int_C2-P _{TEF_{in}} - dlGAS(GGGGS) ERG20-T _{lip2}		dlGAS-GGGGS-R	ggacatagaaccaccaccaccgatgccctt cagagagggc
		GGGGS- ERG20	GGGGS-ERG20-F	ggtggtggtggttctatgtccaaggcgaatt cga
			GGGGS-ERG20-R	gttgattccgaacagaagctacttctgtcgctt gtaaadc
		ERG20- GGGGS	ERG20-GGGGS-F	ttgcagtactaaccgcagccaaggcgaat tcgaaagc
11	Int_C2-P _{TEF_{in}} - ERG20(GGGGS) dlGAS-T _{lip2}		ERG20-GGGGS-R	tcgatagaaccaccaccaccttctgtcgc ttgtaaadc
		GGGGS- dlGAS	GGGGS-dlGAS-F	ggtggtggtggttctatgcaccatcacct gttc
			GGGGS-dlGAS-R	gttgattccgaacagaagttagatgccctca gagagg
			ERG20-F	ttgcagtactaaccgcagccaaggcgaat tcgaaag
12	Int_C2-P _{TEF_{in}} - dlGAS-T _{lip2} - P _{TEF_{in}} -ERG20- T _{lip2}	ERG20	ERG20-R	tgattccgaacagaagctacttctgtcgttgt aaadc
For enhancing the flux of germacrene A synthetic route				
			P _{HMG1} -up-F	cgcgacaggtgatcgaatc
		P _{HMG1} -up	P _{HMG1} -up-R	ccccaaccggctcgtatctgtgccttc gtggtc
13	Int-P _{TEF_{in}} -HMG1	P _{TEF_{in}} -HMG1	P _{TEF_{in}} -HMG1-F	aatcgagaccgggttgccggcgt
			P _{TEF_{in}} -HMG1-R	gtagctcggttagtactgcaaaaagtgc
			P _{HMG1} -dw-F	tttgcagtactaaccgcagctacaagcagct attggaagattg
		P _{HMG1} -dw	P _{HMG1} -dw-R	gatggccttgcaacctgc
14	Int-P _{TEF_{in}} -IDI1	P _{IDI1} -up	P _{IDI1} -up-F	gatgagagttgagagagggc
			P _{IDI1} -up-R	atctcacctacgattccac

		$P_{TEF_{in}}-IDI1$	$P_{TEF_{in}}-IDI1-F$	gaaatcgtaggtgaaaatgagaccgggttg gcggcgta
			$P_{TEF_{in}}-IDI1-R$	cgctcgcggttagtactgcaaaaagtgc
		$P_{IDI1-dw}$	$P_{IDI1-dw-F}$	ttgcagtactaaccgcagacgacgtctaca gcgac
			$P_{IDI1-dw-R}$	tggccggtatcaattggtgc
		$P_{ERG13-up}$	$P_{ERG13-up-F}$	cctcggcattttgactcacaagttc
			$P_{ERG13-up-R}$	caaatacggccaaccgggtctcgagtgg tgacatgaacaaggc
15	Int- $P_{TEF_{in}}-ERG13$	$P_{TEF_{in}}-ERG13$	$P_{TEF_{in}}-ERG13-F$	ctcgagaccgggttgccggcg
			$P_{TEF_{in}}-ERG13-R$	cgactgcggttagtactgcaaaaagt
		$P_{ERG13-dw}$	$P_{ERG13-dw-F}$	cactttttgcagtactaaccgcagtcgcaacc ccagaacgttg
			$P_{ERG13-dw-R}$	ctgctgtatctcgtactttcgtcg
		$P_{ERG12-up}$	$P_{ERG12-up-F}$	aggtcgccttttggtattggtgttg
			$P_{ERG12-up-R}$	cggcccaaccgggtctcgacaggaggaa acctgtggtgta
16	Int- $P_{TEF_{in}}-ERG12$	$P_{TEF_{in}}-ERG12$	$P_{TEF_{in}}-ERG12-F$	gagaccgggttgccggcgat
			$P_{TEF_{in}}-ERG12-R$	gtcctcgcggttagtactgcaaaaa
		$P_{ERG12-dw}$	$P_{ERG12-dw-F}$	ttgcagtactaaccgcagactacatcattc ggcgccaggc
			$P_{ERG12-up-R}$	ttctcctccgtccgacttgtgctc
		$P_{ERG8-up}$	$P_{ERG8-up-F}$	ttctcctccgtccgacttgtgctc
			$P_{ERG8-up-R}$	aggacatcctactgcgtcttaaggttggtg ccagag
17	Int- $P_{GPD}-ERG8$	$P_{GPD}-ERG8$	$P_{GPD}-ERG8-F$	ttaagacgcagtaggatgtctcgc
			$P_{GPD}-ERG8-R$	tcattgtgatgtgtttaaattc
		$P_{ERG8-dw}$	$P_{ERG8-dw-F}$	attaaacacacatcaacaatgatcttcgaccg gctcttc
			$P_{ERG8-dw-R}$	tgtgatttgccttcatggc
		$P_{ERG19-up}$	$P_{ERG19-up-F}$	catgataaaggctccgattc
			$P_{ERG19-up-R}$	aggacatcctactgcgtctgggtggtgtaac ctttttttc
18	Int- $P_{GPD}-ERG19$	$P_{GPD}-ERG19$	$P_{GPD}-ERG19-F$	ccagacgcagtaggatgtctcgc
			$P_{GPD}-ERG19-R$	cattgttgatgtgtttaaattc
		$P_{ERG19-dw}$	$P_{ERG19-dw-F}$	attaaacacacatcaacaatgatccaccagg cctccac
			$P_{ERG19-dw-R}$	cactccactcacaagacctc
		$P_{ERG9-50bp-up}$	$P_{ERG9-50bp-up-F}$	gttgtcgatataggctgagc
19	Truncated- $P_{ERG9-50bp}$		$P_{ERG9-50bp-up-R}$	atattagcaacacgatctac
		$P_{ERG9-50bp-dw}$	$P_{ERG9-50bp-dw-F}$	agatcgtgttgctaataatggacacaaactcg ctgttc

			P _{ERG9-50bp-dw} -R	cgaaaatctctcggggtgac
			P _{ERG10-up} -F	ccacctgcgaaatcatgac
		P _{ERG10-up}	P _{ERG10-up} -R	caaatacgccccaacccgggtctcagagg
				gaacacgacgatgac
20	Int-P _{TEFin} -ERG10	P _{TEFin} -ERG10	P _{TEFin} -ERG10-F	ctctgagaccgggtggcggcgta
			P _{TEFin} -ERG10-R	gctcctcggttagtactgcaaaaagtgc
		P _{ERG10-dw}	P _{ERG10-dw} -F	cacttttgcagtactaaccgcaggagcccc
				tctacattgttctactgc
			P _{ERG10-up} -R	cagaccgtctcgctggatac
For optimizing copy numbers of dlGAS				
		Int_C3-up	Int_C3-up-F	cattcatgttagttgcgttacagtgtctatcaa
				c
			Int_C3-up-R	aggaaaggagaactgaggtc
		C3-P _{TEFin}	C3-P _{TEFin} -F	ggcgagaccgggtggcggcgta
			C3-P _{TEFin} -R	gtcgtcggttagtactgcaaaaagtgc
			C3-dlGAS-F	tttgcagtactaaccgcagcagaccatcacc
21	Int_C3-P _{TEFin} -dlGAS-T _{pex20}	C3-dlGAS	C3-dlGAS-F	tgttcttcg
			C3-dlGAS-R	acttccccatccacttttagatgcccttcag
				agagg
		C3-T _{pex20} -	T _{pex20} -F	aaaagtgtggatggggaagtga
			T _{pex20} -R	gtaacgcaactaatgaatgaatac
			Int_C3-dw-F	gtgtatcatatcgaccaac
		Int_C3	Int_C3-dw-R	cgccccaacccgggtctcgccatagcacta
				ttgtagag
			Int_D1-up-F	aaggtttgggacgctcgacattgctctaac
		Int_D1-up	Int_D1-up-R	actctg
			Int_D1-up-R	tgaattgagcagcttggtac
		D1-P _{TEFin}	D1-P _{TEFin} -F	cagagagaccgggtggcggcgta
			D1-P _{TEFin} -R	gtcgtcggttagtactgcaaaaagtgtgc
				gtc
			D1-dlGAS-F	cacttttgcagtactaaccgcagcagccat
22	Int_D1-P _{TEFin} -dlGAS-T _{CYC1}	D1-dlGAS	D1-dlGAS-F	cacctgttcttc
			D1-dlGAS-R	attacatgattagatgcccttcagagagg
		T _{CYC1}	D1-T _{CYC1} -F	ggcatctaatactgtaattagttatgtca
			D1-T _{CYC1} -R	gtccgagcgtccaaaaccttctc
			Int_D1-dw-F	tcttcttccccacacatc
		Int_D1-dw	Int_D1-dw-R	cgccccaacccgggtctctctgctgtgctg
				tttgtgc
			Int_E1-up-F	gaaggtttgggacgctcgaagcactatcct
23	Int_E1-P _{TEFin} -dlGAS-T _{CYC1}	Int_E1-up	Int_E1-up-R	ctgctgag
				gggggagcttaatatgatgc
		E1-P _{TEFin}	E1-P _{TEFin} -F	gacacaagagaccgggtggcggcgta

			E1-P _{TEFin} -R	gtcgtgcggtagtactgcaaaaagtctg gtc
		E1-dIGAS	E1-dIGAS-F	tttgcagtactaaccgcagcgacctacc tgttcttcgac
			E1-dIGAS-R	attacatgattagatgcccttcagagggc gggcatctaatacatgtaattagttatgcacgc
		E1-T _{CYC1}	E1-T _{CYC1} -F	t gtgcttcgagcgtccaaaaccttctca
			E1-T _{CYC1} -R	ccgtctctctacagattc
		Int_E1-dw	Int_E1-dw-F	cgccccaaccggctcttctgtcgaata caacagc
			Int_E1-dw-R	
			Int_E3-up-F	ccaatggatgcgctattc
		Int_E3-up	Int_E3-up-F	cgccccaaccggctctctgttctgtcttg agtttg
			Int_E3-up-R	
		E3-P _{TEFin}	E3-P _{TEFin} -F	caaacagagaccgggtggcggcgta tgggtcgtcggtagtactgcaaaaagtg
			E3-P _{TEFin} -R	ctg
			E3-dIGAS-F	tttgcagtactaaccgcagcgacctacc tgttcttcgac
24	Int_E3-P _{TEFin} - dIGAS-T _{lip2}	E3-dIGAS	E3-dIGAS-F	ttccgaacagaagttagatgcccttcagaga gggc
			E3-dIGAS-R	ctgaagggcactaactctgttcggaatcaa cctc
		E3-T _{lip2}	E3-T _{lip2} -F	gtgccagacagatgcattctggcggtc cgccaagaatgcatctgtctggcacagaa
			E3-T _{lip2} -R	atgaccac
		Int_E3-dw	Int_E3-dw-F	gagaatagcaacgaggtctc
			Int_E3-dw-R	
For regulating lipids metabolism				
			S667-up-F	taacctcacaccactc
		S667D-up	S667D-up-F	ccaccgtcgtcaagaggctactccaatgt cg
25	ACC1 ^{S667D}		S667D-up-R	
		S667D-dw	S667D-dw-F	gacctcttgacgacgggtattctgtgtct
			S667-dw-R	agagaagacacctgatgacg
			S667-up-F	As above
		S667E-up	S667E-up-F	ccaccgtcctcaagaggctactccaatgt cg
26	ACC1 ^{S667E}		S667E-up-R	
		S667E-dw	S667E-dw-F	gacctcttgaggacgggtattctgtgtct
			S667-dw-R	As above
			S1178-up-F	gagtcggagccaagaacaac
		S1178D-up	S1178D-up-F	tgggagacgtcatcagctcgagaccgag g
27	ACC1 ^{S1178D}		S1178D-up-R	
		S1178D-dw	S1178D-dw-F	gagctgatgacgtctccactttctgacac

			S1178-dw-R	acgtggatgtttcggttgc
			S1178-up-F	As above
28	ACC1 ^{S1178E}	S1178E-up	S1178E-up-R	tcggagacctcatcagctcgagacaccgagg
		S1178E-dw	S1178E-dw-F	gagctgatgaggctccgactttctgtacac
			S1178-dw-R	As above
		Int_E3-AC-up ^c	Int_E3-AC-up-F	tagttgcgttgttgatgcttgagttgagg
			Int_E3-AC-up-R	taactttttcgtgactctgttctc
		P _{EXP1} ^c	P _{EXP1} -F	aaggccactaaggagttggcggcggttt
			P _{EXP1} -R	tgctgtagatatgtcttgtgtg
			E3-T _{pex20} -F	aagtgtggatggggaagtgag
		E3-T _{pex20} ^c	E3-T _{pex20} -R	catcaaacacgcaactaacatgaatgaatac
		Int_E3-AC-dw ^c	Int_E3-AC-dw-F	aaactccttagtggccttctggcacagaa
			Int_E3-AC-dw-R	gtgaaggaaatgcctaaacctg
29	Int_E3-P _{EXP1} -MFE1-T _{pex20}	MFE1	MFE1-F	caagacatatctacagcaatgctcggagaac
			MFE1-R	taagatacgac
				acttccccatccacacttttagagcttagcatc
				cttggg
30	Int_E3-P _{EXP1} -FAA1-T _{pex20}	FAA1	FAA1-F	caagacatatctacagcaatggcggataca
			FAA1-R	caatttcc
				acttccccatccacacttctaagactgctcgt
				agcaact
31	Int_E3-P _{EXP1} -POT1-T _{pex20}	POT1	POT1-F	caagacatatctacagcaatggaccgactta
			POT1-R	acaacctc
				acttccccatccacacttttactcggcaacaa
				ccagag
32	Int_E3-P _{EXP1} -PEX10-T _{pex20}	PEX10	PEX10-F	caagacatatctacagcaatgtggggaagtt
			PEX10-R	cacatgc
				ttccccatccacacttttatctgataggcaaca
				agttctgc
33	Int_E3-P _{EXP1} -TGL4-T _{pex20}	TGL4	TGL4-F	caagacatatctacagcaatgttcactcca
			TGL4-R	gagtttcc
				acttccccatccacacttttagcacgagtcag
				aacagttc

^aThe common fragments used for evaluating the efficiency of GASs and fusing dIGAS and ERG20.

^bPromoter and terminator sequence of gene XXX were represented by P_{XXX} and T_{XXX}, respectively.

^cThe common fragments used for regulating lipids metabolism.

Table 4. List of primers used for qPCR in this work.

PCR fragment	Primer name	Sequence (5'-3')
qHMG1	qHMG-F	catacctcgccatcctcgac
	qHMG-R	atgtgggtccaatcagcagg
qERG13	qERG13-F	ggtgagggtcgatcttggg
	qERG13-R	aatgagcatggcaacacagc
qIDI	qIDI-F	catgaacacggaaaaggccc
	qIDI-R	atctgtgtgtgctggactg
qERG12	qERG12-F	ctcactaaggtccagcaggc
	qERG12-R	atcttcgcacacagaagggg
qERG19	qERG19-F	cccctgattcaccaggac
	qERG19-R	cattttgtgtcgagagccc
qERG8	qERG8-F	accggctcttcaaacacgaa
	qERG8-R	tgctgaatacggcgatcaa
qACT1	qACT1-F	tccaggccgtcctctccc
	qACT1-R	ggccagccatatacgagtgc

Table 5. Synthetic GAS genes and endogenous DNA sequences used in this study.

Name	Sequence (5'-3')
<i>neGAS</i>	atgacccgacgaatcgccgcctctcccttctctgcccagtgaggccatcgccaccacttctgccccaacccaactgat cctagaggcagacctctggggccgtgcccggacagcctgctggcctcgtcctgcccgtggacgccactcagttcgctct tcctctgtctcgcacagcgaatcatgcagctgccccgactggagtgccctatcagccctctgtgacccccgccgatc agatccacgagaacaccgtggagtgggcccaagccatgggcttcgcccgatctcagaagcacatcgacgccctgcgaga ctctcgagtgggccacctgctggcccgaatctccccgccaccgacatggtggccctgcaagtggccgtggactgga ccacctgttctgtctgacgaccgactggagcagatccacggcggctgctggggcccctacctgcaaacctgc tggcgctgtccgagacggcggcccacctcagctgatcacccttcgccaagccttcggcgacctgcgagagcgaatg ctggagctgcgagtgcccaactggatccccgattctcttctgtgtggagcactgttccaagccttcgtggacgagcca agtaccgagccggcggtgggtgccccttcgctctcactctcgaatccgacagatcaccgtgggctgtacaccggct tcctgtggcgagctgaccgacacatctctgcccccgactgctggagcagagctgtgacgacctggagcga tctgcctctaccatcgtggcctggccaacgacatctacaccgtggagaaggagcagccaaggcgaggtgaacaaca ccgtgctggtgctgatgcacgaggacggcctgtcttcgacgacgcctggcccgaaccgtgcagctgcacaacatcgag atgcgagagttcgagcactggtggccgacctgcccctttcagcaggacatcgacgatcagctgcgacgatactgca agtgtgatccactctctggtccacgccgactggggccgatctaccggccgatcagccccggcgacggcgggcg ccgtgcgataa
<i>spGAS</i>	atccccgacatctccacccttccccctaccgaatctctccctacgtggcccgaagcccagagcactgaccgactggacc cgacgaatcgccctgatcgagcagagggcccccgaagcattcgccaagccgacttcggctggttcgccccatgg tgtacccaccggcagccactcagctaacctgatggccgactggttcgctggctgttctctggtggacgatcagctgg acgacggctctatcgccgatctcccagcaagcccagacatcgtggacggaatcgagccgctctggagtctcccgga ccatggcggcctgcccctgcccgatagagacggccctgtggctgtctctgcccggcaccctggtgagaaccgcc ctcagccaccgccaactggcgaagacattcgtgcagcactggacgactgtctgaccgtcggccacttgggagggc cggcaaccgagtgccggcaccgtgcccagcagggagacctacatcgagatcgacgacacaccggcggccatctactg gtgtatgacctgatcgacattgtggagagaatcgacatccccgactctctgtacccgacgctcagttccgagccgctg gacggcctgtaacgtggtgtgtggaccaacgacgtgactctctggccaaggagcagccctggggcaggtgcaca acctggtgcacatcgtggagcaccaccgaggcctggatactcccggagccctggaccacgtgtgtgcccacccttacc gagaccatgctgttctggagaaggaacgagagctctcggcccggaccgagcatcagccgtgctgctgctgctgctg ggccggcatcgcaacctggatcgaggcaacctggactggtctcgtatctaccaagcgataccgacctggaggggcgac gaggagatccgaccgacgagctgaggcctctctgatgaaggtgatcagtaa
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