nature portfolio

https://doi.org/10.1038/s41586-025-09431-y

Supplementary information

Engineered yeast provides rare but essential pollen sterols for honeybees

In the format provided by the authors and unedited

Supplementary Tables

Supplementary Table 1.

Yeasts engineered for non-native sterol production, in previous work and in this study. The highest production levels in each study are given, along with the carbon source used during the corresponding cultivation.

Sterol	Host	Genotype *giving highest content only	Carbon source	Content/titre	Reference
Epi-campesterol ('ergosta-5-eneol')	*S. cerevisiae* FY1679-28C (S288C derivative)	*AtDHCR7*	Glucose/ethanol/ galactose	45% w/w total sterol	55
Epi-campesterol ('ergosta-5-eneol')	*S. cerevisiae* FY1679-28C (S288C derivative)	*erg5Δ* *AtDHCR7*	Glucose/ethanol/ galactose	0.3 mg/l/OD (free sterol only)	56
Epi-campesterol	*S. cerevisiae* RH2881	*erg5ΔDrDHCR7*	Glucose (2% w/v)	98% w/w total free sterol Total sterol: 3.0 ug/10^7 cells	57
Epi-campesterol	*Y. lipolytica* ATCC201249	*erg5Δ* *XlDHCR7*	Sunflower seed oil (batch: 3.7% v/v, then maintained < 2g/L)	453 mg/L, inferred content: 20 mg/g DCW (5-L bioreactor, fed-batch)	58
Campesterol	*S. cerevisiae* BY4742	*erg4Δerg5ΔArDWF1* (plasmid)	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	59
Epi-campesterol	*Y. lipolytica* ATCC201249	*erg5ΔDrDHCR7* *↑POX2*	Sunflower seed oil (batch: 28.86 g/L, then maintained < 2g/L)	942 mg/L, inferred content: 36 mg/g DCW (5-L bioreactor, fed-batch)	60
Epi-campesterol	*Y. lipolytica* PO1f (ATCC No. MYA-2613)	*erg5Δmfe1Δ* *XlDHCR7* (2x)	Glucose (batch: 40 g/L, maintained >10 g/L by feeding 50% glucose solution)	837 mg/L, inferred content: 32 mg/g DCW (5-L bioreactor, fed-batch)	61
Campesterol	*S. cerevisiae* CENPK2.1D	*AtDWF7* *AtDWF5* *AtDWF1 ↑ (ERG7* *ERG8* *ERG10* *ERG12* *ERG13* *ERG19* *ERG20* *UPC2* *tHMG1 (3x))*	Glucose (2% w/v)	41 mg/L	62
Epi-campesterol	*S. cerevisiae* YS5	*erg5ΔXlDHCR7*	Glucose (20 g/L)	178 mg/L (3 mL test tube)	63
Epi-campesterol	*S. cerevisiae* BY-T30 (BY4742)	*StDWF5 ↑* *(ERG1* *ERG8* *ERG9* *ERG10* *ERG12*	Glucose (2% w/v)	7.8 mg/L/OD600 (shake flask)	64

		ERG13 *ERG19* *ERG20* *IDI1* *tHMG1)*			
Cholesterol	*S. cerevisiae* RH2881	*erg5Δerg6Δ* *DrDHCR7* *DrDHCR24*	Glucose (2% w/v) Molasses (0.75% v/v)	96% w/w total sterol, 1mg/g wet weight of yeast, 10 mg/L	57
Cholesterol	*P. pastoris* *CBS7435* *Δhis4Δku70*	*erg5Δerg6ΔDrDHCR7* *DrDHCR24*	Glycerol (1% w/v) Methanol (1% w/v)	89% of total sterol (shake flask)	65
Cholesterol	*S. cerevisiae* BY-T3 (BY4742)	*erg6Δatf2Δ* *DrDHCR7* *DrDHCR24* ↑ *(ERG1* *ERG9* *ERG20)*	Glucose (2% w/v) Galactose (2% w/v)	16 mg/L (250 mL shake flask)	66
Cholesterol	*S. cerevisiae* BY-T30 (BY4742)	*StDWF5 GgDHCR24* ↑ *(ERG1* *ERG8* *ERG9* *ERG10* *ERG12* *ERG13* *ERG19* *ERG20* *IDI1* *tHMG1)*	Glucose (2% w/v)	8.5 mg/L/OD600 (shake flask)	64
Cholesterol (intermediate in diosgenin synthesis)	*S. cerevisiae* (BY4742)	*atf2Δ* *StDWF5 GgDHCR24* ↓ERG6 *Vc*CYP90B27 *Dzin*CYP90G6 *Vc*CYP94N1 *Vv*CPR *Vc*CYP90B27 *Vc*CYP94N1 *Vv*CPR ↑ *(ERG1* *ERG8* *ERG9* *ERG10* *ERG12* *ERG13* *ERG19* *ERG20* *IDI1* *tHMG1)*	Glucose (2% w/v) (Fed to 5 g/L)	170 mg/g DCW (shake flask), 5.5 g/L (5-L fermentation)	64
Desmosterol	*S. cerevisiae* RH2881	*erg6ΔDrDHCR7*	Glucose (2% w/v)	87% w/w total free sterol Total sterol: 3.4 ug/10^7 cells	57
Desmosterol	*S. cerevisiae* BY4742	*erg6ΔStDWF5*	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	32
β-Sitosterol	*S. cerevisiae* CENPK2.1D	*erg4Δ* *AtDWF7* *AtDWF5* *AtDWF1* *SMT2 (plasmid)*		2 mg/L	62

		are1Δ *are2Δ* ↑ *(ERG10* *ERG12* *ERG13* *tHMG1)*			
7-Dehydrocholesterol	*S. cerevisiae* RH2881	*erg5Δerg6ΔDrDHCR24*	Glucose (2% w/v)	86% w/w total free sterol, Total sterol: 3.2 ug/10^7 cells	57
7-dehydrocholesterol	*S. cerevisiae* RH2881	*erg5Δerg6ΔGgDHCR24* ↑ *(ERG1* *ERG2* *ERG3* *ERG11* *Erg24* *ERG25* *ERG26* *ERG27)*	Glucose (4% w/v) Galactose (1% w/v)	360.6 mg/L (shake-flask)	38
24-Epi-ergosterol	*S. cerevisiae* CICC1746 (industrial ergosterol production strain)	*ArDWF1* *erg4Δ↑ (ERG5, YEH1, YEH2, ARE2, UPC2)*	Glucose (batch: 10 g/L glucose, feed: 500 g/L) Ethanol	2.76 g/L 19.27 mg/gDCW (2-L bioreactor, fed-batch)	37
24-Methylenecholesterol	*S. cerevisiae* BY4742	*erg4Δerg5ΔStDWF5*	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	32
24-Methylenecholesterol	*S. cerevisiae* YS5	*erg4Δerg5ΔXlDHCR7 (2x)*	Glucose (20 g/L)	225 mg/L (250 mL shake flask)	63
24-Methylenecholesterol	*Y. lipolytica* ST9100 (W29 derivative)	*erg4Δ erg5Δ* *SeACS TtSTC1* *Tsp*DWF7 ↑ *(HMG1, ERG12, ACL1, IDI1, ERG20)*	Glucose (8% w/v)	42.2 mg/g DCW (24-deep well plate)	This study
Desmosterol + cholesterol	*S. cerevisiae* BY4742	*erg6ΔStDWF5* *SlSSR2*	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	32
24-Methylenecholesterol + campesterol	*S. cerevisiae* BY4742	*erg4Δ* *erg5ΔStDWF5* *SlSSR1*	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	32
24-Methylenecholesterol + cholesterol + desmosterol (inferred)	*S. cerevisiae* BY4742	*erg4Δ* *erg5ΔStDWF5* *SlSSR2*	Glucose (2% w/v) Galactose (2% w/v)	Not quantified	32
24-Methylenecholesterol + campesterol	*Y. lipolytica* ST9100 (W29 derivative)	*erg4Δ erg5Δ* *SeACS TtSTC1* *Tsp*DWF7 *StSSR1* ↑ *(HMG1, ERG12, ACL1, IDI1, ERG20)*	Glucose (8% w/v)	20.3 mg/g DCW (24-deep well plate)	This study
24-Methylenecholesterol + campesterol + isofucosterol + sitosterol	*Y. lipolytica* ST9100 (W29 derivative)	*erg4Δ erg5Δ* *SeACS TtSTC1* *Tsp*DWF7 *StSSR1* *CqSMT2* ↑ *(HMG1, ERG12, ACL1, IDI1, ERG20)*	Glucose (8% w/v)	20.3 mg/g DCW (24-deep well plate)	This study

24-Methylenecholesterol + campesterol + isofucosterol + sitosterol + cholesterol + desmosterol	*Y. lipolytica* ST9100 (W29 derivative)	*erg*4Δ *erg*5Δ *SeACS TtSTC1* *T*sp*DWF7* *StSSR1* *CqSMT2* *SlDHCR24* ↑ *(HMG1, ERG12, ACL1, IDI1, ERG20)*	Glucose (8% w/v) (Bioreactor batch media 40 g/L glucose, feed media 300 g/L glucose)	24.6 mg/g DCW (24-deep well plate) 9.11 mg/g DCW (5 L bioreactor)	This study

Supplementary Table 2.
Yarrowia lipolytica strains used in this study. Corresponding genotypes, parental strains and elements used for the construction of each strain are given.

			Elements used to construct strain	
Strain name	**Genotype**	**Parent strain/Reference**	**gRNA vector**	**Integration vector/BioBrick**
ST4842 'W29 strain'	MATa	*Y. lipolytica* W29 (MatA, ATCC©20460™) strain Y-63746 from the ARS culture collection		
ST6512	MATa ku70Δ::PrTEF1->Cas9- TTef12::PrGPD->DsdA-TLip2	ST4842, Marella *et al.* (2020)[67]		
ST9100 'Platform strain'	MATa ku70Δ::PrTEF1->Cas9- TTef12::PrGPD->DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt- >ERG12 IntC_3-SeACS<-PrGPDPrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20	ST6512, Arnesen *et al.* (2020)[23]		
ST11005 'Tet strain'	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC	ST9100	pCfB6637 (pNat-YLgRNA3_IntE_3)	pCfB10494 (IntE3_PrGPAT->TtSTC)
ST11014	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC Δerg5_HphMX	ST11005		BB5098 (Erg5_HphMX_KO)
ST11027	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ	ST11014		pCfB6611 (pNat-PrExp-Cre)
ST11040	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX	ST11027		BB5097 (Erg4_HphMX_KO)
ST11056	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Stuberosum	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10249 (IntE4_PrTEFInt->DHCR7_Stuberosum)
ST11057	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Drerio	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10250 (IntE4_PrTEFInt->DHCR7_Drerio)
ST11058	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Ldrancou	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10251 (IntE4_PrTEFInt->DHCR7_Ldrancou)
ST11059	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Esilicul	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10252 (IntE4_PrTEFInt->DHCR7_Esilicul)
ST11060	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Cprotoch	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10253 (IntE4_PrTEF->DHCR7_Cprotoch)

ST11061	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Csubellip	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10254 (IntE4_PrTEFInt->DHCR7_Csubellip)
ST11062	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Mverticillata	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10255 (IntE4_PrTEFInt->DHCR7_Mverticillata)
ST11063	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Gsoja	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10256 (IntE4_PrTEFInt->DHCR7_Gsoja)
ST11064 '24-MC strain'	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Tsp	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10257 (IntE4_PrTEFInt->DHCR7_Tsp)
ST11065	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Wchondrophi	ST11040	pCfB6638 (pNat-YLgRNA2_IntE_4)	pCfB10258 (IntE4_PrTEFInt->DHCR7_Wchondrophi)
ST11943 'Two-sterol strain'	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Tsp IntF3_PrDGA1->StSSR1	ST11064	pBP8003 (pNat-YLgRNA4-IntF_3)	pCfB10948 (IntF3_PrDGA1->StSSR1)
ST12140 'Four-sterol strain'	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Tsp IntF3_PrDGA1->StSSR1 IntE1_PrGPAT->SMT2_Cq	ST11943	pCfB6633 (pNat-YLgRNA2_IntE_1)	pCfB10851 (IntE1_PrGPAT->SMT2_Cquinoa)
ST12178 'Mixed-sterol strain'	MATa ku70Δ::PrTEF1-Cas9-TTef12::PrGPD-DsdA-TLip2 IntC_2-HMG1<-PrGPD-PrTefInt->ERG12 IntC_3-SeACS<-PrGPD-PrTefInt->YlACL1 IntD_1-IDI1<-PrGPD-PrTefInt->ERG20 IntE_3-PrGPAT->TtSTC erg5Δ erg4Δ_HphMX IntE_4-PrTEFInt->DHCR7_Tsp IntF3_PrDGA1->StSSR1 IntE1_PrGPAT->SMT2_Cq IntE5_PrGPAT->DHCR24_Sl	ST12140	pCfB10783 (pNatYlgRNA-IntE_5)	pCfB10853 (IntE5_PrGPAT->DHCR24_Slycopersic)

Supplementary Table 3.

Codon-optimised sequences of heterologous genes used for strain engineering in this study.

Gene	Nucleic acid sequence, codon optimised for *Yarrowia lipolytica*
Solanum tuberosum delta-7 sterol reductase	ATGGCCGAGTCTCAGCTGGTGCACCCTCCTCTGTTCACCTACATCTCTATGCTGGCCCTGCTGACCCTG GTGCCTCCTTTCGTGATCCTGATGTGGTACACCAACGTGCACGCCGACGGCTCTGTGCTGCAGACCTT CAACTACCTGAAGGAAAACGGCCTGCAGGGCCTGATCGACATCTGGCCCCGACCTACCGCCATTGCC GGAAAGATCATCATCTGCTACGCCCTGTTCGAGGCTACCCTGCAGCTGCTGCTGCCCGGCAAGCGAGT GCAGGGCCCCATCTCTCCCACCGGCCACCGACCTGTGTACAAGGCCAACGGCATGGCCGCCTACACC GTGACTCTGATTACCTACCTGTCTCTGTGGTGGTTCGGCATCTTCAACCCCACCGTGGTGTACGACCA CCTGGGCGAGATCCTGTCTACCCTGAACTTCGGCTCTCTGATCTTCTGCCTGTTCCTGTACATCAAGGG ACACGTGGCTCCCTCTTCTACCGACCACGGCTCCTCTGGCAACATCATCGTGGACTACTACTGGGGCA TGGAACTGTACCCTCGAATCGGCAAGCACTTCGACATCAAGGTGTTCACCAACTGTCGATTCGGCATG GTGTCTTGGGGACTGCTGCCCATCACCTACTGCATCAAGCAGTACGAGGAATACGGATCTCTGTCTGA CTCCATGCTGATCCACGCCATCATCACCCTGGTCTACGTGACCAAGTTCTTCTGGTGGGAGGCCGGCT ACTGGAACACCATGGACATTGCCCACGACCGAGCCGGCTTCTACATCTGCTGGGGCTGCCTGGTGTTC CTGCCTTGCATGTACACTTCTCCCGGCATGTACCTGGTGAAGCACCCCGTGAACCTGGGACCTCAGCT GGCCATCTCCATCCTGGTGGCCGGCATCCTGTGCGTGTACATTAACTACGACTGCGACCGACAGCGAC AAGAGTTCCGACGAACTAACGGCAAGGCCCTGGTGTGGGGCAAGGCTCCCTCCAAGATCGTGGCCTC TTACACCACCACCACTGGCGAGACTAAGTCCTCTCTGCTGCTGACCTCCGGCTGGTGGGGCCTGTCTC GACACTTCCACTACGTGCCCGAGATTCTGGCCTCTTTCTTTTGGTCTGTGCCCGCTCTGTTCAACCACA TTATGCCCTACTTCTACGTGATCTACCTGACCGGCCTGCTGCTGGACCGAGCCAAGCGAGATGACGAA CGATGCAAGTCTAAGTACGGCAAGTACTGGAAGAAGTACTGCGAGAAGGTCCCCTACCGAGTGATCC CCGGCATCTACTAA
Danio rerio delta-7 sterol reductase	ATGATGGCCTCTGACCGAGTTCGAAAGCGACACAAGGGCTCTGCTAACGGTGCTCAGACCGTTGAGA AGGAACCCTCCAAGGAGCCCGCCCAGTGGGGCCGAGCCTGGGAGGTCGATTGGTTCTCCCTGTCCGG CGTGATTCTGCTGCTGTGCTTTGCCCCTTTCCTGGTCTTCTTCTTCATCATGGCTTGTGATCAGTACCAG TGCTCTATCTCCCATCCCCTTCTGGACCTTTACAACGGTGACGCCACTCTGTTCACCATCTGGAACCGA GCCCCCTCCTTCACCTGGGCCGCTGCCAAGATCTACGCCATCTGGGTCACCTTCCAGGTCGTTCTGTA CATGTGCGTCCCCGACTTCCTGCACAAGATCCTGCCAGGTTACGTCGGCGGTGTCCAGGACGGAGCTA GAACTCCCGCCGGCCTGATCAACAAGTATGAGGTTAACGGTCTGCAGTGCTGGCTCATCACCCACGT GCTCTGGGTGCTGAATGCTCAGCACTTCCACTGGTTTTCACCCACCATTATCATTGACAACTGGATCC CCCTGCTGTGGTGCACCAACATTCTGGGCTATGCCGTCTCCACCTTCGCTTTCATCAAGGCCTACCTGT TCCCCACCAATCCCGAGGACTGCAAGTTCACCGGAAACATGTTTTACAATTACATGATGGGTATTGAG TTCAACCCCCGAATCGGTAAGTGGTTCGACTTCAAGCTGTTCTTCAACGGTCGGCCTGGCATCGTCGC CTGGACCCTCATCAACCTTTCCTACGCTGCTAAGCAGCAGGAGCTGTACGGCTACGTCACCAACTCTA TGATCCTGGTCAACGTCCTGCAGGCCGTGTACGTTGTCGACTTCTTCTGGAACGAGGCTTGGTACCTG AAAACCATCGACATCTGCCACGACCACTTTGGCTGGTACCTGGGATGGGGAGACTGCGTTTGGCTGC CTTTCCTGTACACCCTGCAGGGTCTGTACCTGGTCTACAACCCTATCCAGCTGTCCACTCCCCACGCTG CCGGCGTGCTGATCCTGGGTCTGGTCGGTTACTACATTTTTCGAGTGACCAACCACCAGAAGGACCTC TTCCGACGAACTGAGGGCAACTGTTCGATCTGGGGCAAGAAGCCGACCTTTATCGAGTGCTCCTACC GATCTGCCGACGGCGCCATCCACAAGTCCAAGCTTATGACCTCCGGCTTTTGGGGTGTTGCCCGACAC ATGAACTACACTGGTGACCTTATGGGTTCCCTGGCCTACTGTCTGGCCTGTGGTGGTAACCACCTCCT CCCCTACTTCTACATTGTCTACATGACTATTCTGCTGGTCCACAGATGCATTCGAGACGAGCACCGAT GCTCCAACAAGTACGGCAAGGATTGGGAACGATACACCGCCGCCGTCTCTTACCGACTGCTGCCCAA CATCTTCTAA
Legionella drancourtii delta-7 sterol reductase	ATGTACTTCAAGATCCGAAACACCCTGGGACCTCTGCTGCTGATCCTGTCTTGCCCCATCTTCGTGAT GCTGATGTGGTACACCAACACCGAGCTGAAGGGATCTCTGTCTACCCTGTGGGACCTGATCGTGCAG CAGGGCCTGTTCGAGACTACCTACAAGATCTGGCAGCCCTACTTCTGGGGCTCTGCCCTGGCCTGGAA GGTGATCTTCGCCTTCATCATCTTCGAGCTGGCCCTGATGCGACTGCTGCCCGGCAAGGAATTCACCG GACCTGTGACTCCCAAGGGCAACGTGCCCATCTACAAGGAAAACGGACCCCTGGCCTTCATTACCAC CATGACTACCTTTTGCGTGGCCTCTTTCGGCCTGCATCTGTTCCCCGCCTCTATCCTGTACGACAACCT GGGCGCCATCCTGGGAGCCCTGAACGTGTTCTCTCTGATCTTCTGCGCCCTGCTGTATATCAAGGGCC GATACTTCCCCTCTTCTACCGACTCTGGCATCACCAACAACATCATTTTCGACTACTACTGGGGAACC GAGCTGTACCCTCACATCTTCGGATGGTCTATCAAGAAGTTCATCACCTGTCGATTCGGCATGATGTC TTGGGGACTGTTCCTGATCTCTTACTGCGCCAAGCAGGCCGAGCTGGGCGACCTGGCCAACTCTATGC TGATCTCTGTGGCCCTGCAGTTCCTGTACCTGTCTAAGTTTTACCTGTGGGAGAAGGGCTACCTGCGA TCTCTGGACATTATGCACGACCGAGCCGGCTTCTACATCTGCTGGGGCTGCCTGGTGTGGGTGCCCTG CATCTACACTTCTCCCTCTATGTACCTGGTGCTGCACCCCATCCACCTGTCTTTCTGGCTGGCCACCTC CATTCTGGTGCTGGGAGCCGCTTCCATCCTGATCAACTACTTCGCCGACCGACAGCGACTGATGACCC GAGCCACCGACGGCGAGTGTAAGATCTGGGGAAAGAAGCCCGTCACCGTTTTCGCCCAGTACCAGAC CACCGAGGGCGACAAGAAGCAGACCATCCTGCTGGCCTCTGGCTGGTGGGGCGTCGCCCGACACTTC CACTACGTGCCCGAGCTGGCTGGAACCTTCTTCTGGTCTGTGCCCGCTCTGTTTGAGAACTTCTCTCCT TACTTCTACCTGTGCTTCCTGACCATTCTGCTCGTGGACCGAGCCTTCCGAGATGACCGACGATGCTCT

	GACAAGTACGGACAGTACTGGCACAAGTACTGCGAGCTGGTGCCTTACAAGATCGTGCCCTTCGTGA TCTAA
Ectocarpus siliculosus delta-7 sterol reductase	ATGATCGACGGCGCTGCCATCGGACGATCTCCCGTGATCTCTTCTTGGCACGGCTACAACCCCGCCTC TTCGCAGCAGCAGTGTCTGCAGGCCGTCCAGACCTCTCTGCCCACCACCGACGGCGACCGAGAGCGA CGACGATCTATGGCCCTGCGAACCTCTACCAAGCAGGCCTCTGACGCCATGGACATCCGAGAGGCCG TGAAGGCCTCTGTGGCCGCCGAGTCTAAGGACTCTAAGCTGTGGGGCTTCGTGCCCAACTGGTTCCGA ACTACCGTGGGACCCCTGTTCCTGATCCTGGTGCCTCCTTTCTTCGTGGTGTTCTGGTGGCACCTCCTG GTGTCTCACTCTGGCTCTTGGGTGTCTCTGTGGGGCGACCTGAAGGCCGCTGGACCCGAGTACGTGCT GGACGTGGTGCCCTCTCCTGTGGACCCCGCTGCCTGGAAGTACATCCTCGGCTTCGGCGTGTTCGAGA TCCTGCTGATGGTGGGACTGCCCGGCAAGGCCTTCCGAGCTAACCCCACCGCCACCGGACACATCCC CGTGTACAAGGCCAACGGCATGCTGTCTTACCTGGTGACCCTGGCTACCCTGTGCGCCCTGGTCGCCA CCGACCGACTGGACCCCAAGAACGTGTACGACAAGCTGGGCGAGATCTTCACCGGCCTGTCTGTGTT CTCTCTGTTCTTCGTGCTGCTGCTGACCGTGAAGGGCCTGTACTTCCCCTCTACCGACGACTCTGGATC TAACGGATCTTTCCTGCAGAACTACTGGTGGGGCACCGAGCTGTACCCTCGAGTGTTCGGAGCCGAC GTGAAGATGTTCACCAACTGCCGATTCGGCATGATGTACTGGGCCGTGGGCGCCGTGATCTACGCTTA CACCCAGCAGCAGATGTACGGAAAGCTGTCCTCTTCTATGGCCGTGTCTGTGATCCTGCAGCTGACCT ACATCACCAAGTTCTTCCACTGGGAGATGGGCTACATGAACTCTATGGACATTCAGCACGACCGAGC CGGCTACTACCTGTGCTGGGGCTGCCTGGTGTGGGTGCCCGCCGTGTACTCTTCTCCCGGCATCTACC TGGTCAAGCACCCCATTTCTCTCGGCTGGTACGGCGCCTCTGCCATTCTGGCCCTGGGCCTGCTGTCTA TCTGGGCCAACTTCGACGCTGACCGACAGCGACACGCCTTCCGACAGGCCAAGGGCGACATCATCGT GTGGGGCAAGCCCGCCAAGTACATCACCGCCGGCTACATCAACGCCCGAGGCGAGAAGGCCTCCTCT CTGCTGCTCTGCACCGGCTGGTGGGGAGTCGCCCGACACTTCCACTACCTGCCTGAGATTACCGGCGC CTTCTTCTGGACCGTGCCTGCTCTGTTCGAGACTCCCACTCCTTACTTCTACGTCGTGTTCCTGGTGCT GCTCCTCACTGACCGAGCTTTCCGAGATGACACCCGATGCCGAGGCAAGTACGGCAAGCACTGGGAC AAGTACTGCGCTCAGGTGCCCTACAAGATCGTGCCCGGCATCCTGTAA
Candidatus *Protochlamydia amoebophila* delta-7 sterol reductase	ATGCTGATCGAGATGCTGTGCATCACCAAGCACATCCTCGAGAAGATCTCTAAGTTCTCTCTGGCTCG ACCCCACGTGGCCAACACCAACATTTTCTTCAGACAGACCTTCGGACCCCTGTTCCTGCTGCTGCTGT GCCCTCCTACCGTGTTCGCCTTCTGGTACACCAACACCTACCTCGAGGGATCTCTGTTCCGATTCACCG AGTTCGCCTGGCAGCAGGGCTTCCTGTCTACCCTCAAGACTATCTGGTTCCCCTACTTCTTCGGCACCT CTATCGCCTGGACCATGCTGGCCATCTTCGCTTCTCTGCAGCTGATCCTGATGCGAATTCTGCCCGGC GAGTGCTACGAGGGCCCCATCACTCCCACCGGACACGTGCCCCTGTACAAGGCCAACCGATTCTCTG CCTTCATCGTGACCGTGTCTATCTTCCTGATCGCCTCTTGCTACTACCAGCTGTTCGCTCCCACCATCA TCTACGACAACTTCCCCGGCCTGCTGGGCGCCCTGAACATCTTCTCCCTGTGTTTCTGCTTCTTCCTGG TGCTGAAGGGCCACTACTTCCCTTCTAACGGCGACGTCGGCGGCTCTGGCAACATCATCTTCGACTAC TACTGGGGCATGGAACTGTACCCTCGACTGCTCGGCTGGGACATCAAGCAGTTCACCAACTGCCGATT CGGCATGATGTCTTGGGCCCTGATCGTGATCTCTTTCGCCGCCAAGCAGCAACAGCTGGACGGCCTGT CTGACTCTATGTTCGTGGCCGTGGCTCTCCAGCTCATCTACATTACCAAGTTCTTCATCTGGGAGCCCG GCTACCTGCGATCTCTGGACATTATGCACGACCGAGCCGGCTACTACATCTGCTGGGGCTGCCTGGTG TGGGTGCCCGGCATCTACACTTCTCCCACTCTGTACCTGGTGGATCACCCCAACCACCTGGGACTCGC CCTGTCCTCTCTGCTGTTCGTGGTGGGCGTGATCGGCATCCTGGTGAACTACCTGGCCGACCGACAGC GACAGCTCGTGCGAAAGAACCAGGGCAACTGTCGAATCTGGGGAAAGGAACCCATCCTGACCATTGC CAAGTACACCACTCAGACCGGCGAGACTAAGCAGAACCTGCTGCTCGCCTCTGGCTGGTGGGGCCTG TCTCGACACTTCCACTACCTGCCTGAGCTGCTGGGAGCCTTCTGCTGGTCTGCTCCCGCTCTGTTCGAG AACTTCCTGCCTTACTTCTACTTCGTGTTTCTGACCCTGCTCCTGACCGACCGAGCTTTCCGAGATGAC CAGAGATGCTCTAAGAAGTACGGCGAGGACTGGAAGATCTACTGCCAGCGAGTGCCCTACAAGATCA TCCCCTTCGTGATCTAA
Coccomyxa subellipsoidea delta-7 sterol reductase	ATGGTCACAACCCGAGCCGCTGCTCGAGCTCAGACCCCTCTGGGCAAAGCTCCTCCCTCCGACCTGTC CCACTCCGAACCCTCCGTCTCTACTCAGAACGGCAAGAAGACATGGGCCGAGACCGCTGGGGCAGGC GAGCACATCGGCGCCTGGGGTATCGGCGGCACTGCCGGACACGTCTTGGCCTACCTGGGTACTCTTGC CCTTATGATCGGTTGCCCCGCCTTTGCAATCTACATGTGGTTTACCCTCACCCACTTGGATGGCTCTCT TGTGGAGCTCGTCCAGTTCGCCCAGAAGGCTGGATTTCAGGGCGTCCGAGCCTCATGGCCCTGGCCCT CCCAGGAGGCCTGGGCCATCATCGCCTCCTTCGGCGGTCTTCAGGCCTTCCTGCAGCTTGCCTTGCCC GGTGCTGTGCACAAGGGCCCAGTCTCTCCCAAGGGTAACGTCCCCGTGTACAAGGCCAACGGTGTCC TCGCCTACTTTACCACCCTGGCTCTGTTCGTCCTTGGCTGGCAGTTCAAGCTGTTTTCCCCCGCCAGAG TCTACGACCTGTTCGGCGAGATCCTTTCCGGCCTGAACATGTTCTCTCTGCTGTTCTGCCTGTTCCTGT ACTTCAAGGGCAAGTACGCCCCCTCATCCTCGGATTCTGGTTCTACCGGCTCCCTGATGTACGATTAT TACTGGGGGATGGAGCTGTACCCTCGGATTGGACGACACTTTGACCTGAAGACTTGGACTAACTGCC GAATGGGCATGATGGGCTGGGGAGTTCTGGTCCTGTGTTACGCTGTGAAGCAGCACGAGCTGTACGG TTACCTTTCTAATTCGATGGCTGTTTCTATCCTGCTCATGCATTTATACATTTTCAAGTTCTTCCTCTGG GAGACTGGTTACTGGGGTACCATGGACATCGCTCACGACCGCGCTGGATACTACCTCTGCTGGGGAT GCCTGAACTGGGTCCCCGCTATCTACACCTCTCCCGCCCTGTACCTCGTCGAGAACCCTATTCAGTGG TCTCTGCCCGCCGCCACCGCTATCGCTGTTGCCGGTACCCTGGCTATCTACATCAACTACGACTCCGA TCGGCAGCGACAGGTTTTCCGAGCTACCAATGGTAAGGCCCTGGTGTGGGGCAAGCCTCCCCAAATT ATCTCTGCCAAGTACATCACCGGCGATGGCAAGCAGAAGACCTCCCTGCTCCTGGCCTCTGGCTGGTG GGGGCTCGCCCGACACTTCCACTACCTGCCCGAGATCTTGGCCGCCTTCTTTTGGACCCTCCCCGCTG

	GTATCTCCCACGCTCTGCCCTACTTTTACGTCTTCTTCCTGACTCTGCTCCTTACCGATCGAGCTTTCCG AGACGACGTCCGATGTAGCTCCAAATACGGTGCCTACTGGCAGCAGTACACCAAGGCCGTTCCCTAC AAGATGATCCCTTACATTTTCTAA
Mortierella verticillate delta-7 sterol reductase	ATGGCCGTGCAGCAGCGAAAGACCCCTGCTCAGGTGGACGTGAAGGCCGAGTCTAAGCTGGACGCCC AGGTGGGCAAGACCTGGGGCCGAGATCGAGATGTGTCTTTCGGCACCATCCTGATCTCCCTGGGCAT CCTGGTGATGTCTCCCATCTGGGTGATGTACACCTACATCTCTTGCAACGCCTACCAGTGCGCCATGT CTGCTCCCGCTCTCGAGATCTACAACTCTCCCGACACTCTGGCCGCCATTCAGACCCTGCTGCTGCGA GAGGTGCCCCGATTCTCTCCCTACGCCGCTCGACTGTTCTTCACCTGGCTGGCCTTCCAGGCCGCTCTG TACGCCTTCCTGCCTGCTCAGATCGGCTACGGCCAGCGAACCCCTGCCGGCCACATTCTGCCCTACAA GGTGAACGGCCTGCTGGCCTGGTTCATCTCTCACTCTATCTACGCTGCCGGCGGACTGTACTTCGGCT GGTGGAAGCTGTCTATCATCCACGACAACTGGGGCGGACTGCTGGTGGCCGCCAACATGTACGGCTA CTTTCTGACATTCTTCTGCTTTATCAAGGCCTACACCTTTCCTTCTCACCCCGCCGACCGAAAGTTCTC TGGCTCTTTCATCTACGACCTGCTGATGGGCATCGAGTTCAACCCTCGAATCGGCAAGCTGTTCGACT TCAAGCTGTTTCACAACGGACGACCCGGCATCGTGGCCTGGACCATGATCAACCTGTCCTTCGCCGCT GCTCAGTACGAGAAGATCGGATACGTGACCAACTCTATGATCCTGCTGAACCTGCTGCATGCTACCTA CGTGCTGGACTTCTTCTACAACGAGGACTGGTATCTGCGAACCATCGACATTGCCCACGACCACTTCG GCTTCTACCTGGCCTGGGGCGACTCCGTGTGGCTGCCCTGGCTGTACACCCTGCAGTCTCACTACCTG GTGCGAAACCCCGTGGACCTGACTCCTGTGCAGTTCGCCTTCGTGTTCACCGTGGGCTACATCGGCTA CTTCATCTTCCGATCTGTGAACCACCAGAAGGACATCGTCCGATCTACCAACGGCGAGTGCATGATCT GGGGCAAGCCCGCCAAGGTGATCCGAACCTCTTTCGTGACCTCTGACGGCAAGACCCACAAGTCTCT GCTGCTGTGCTCTGGCTACTGGGGCCTGTCTCGACACTTCAACTACGTGGGCGACCTGCTCATCTCTCT GGCCATGTGCATGACCTGCGGCACCCAGCATCTGCTGCCCTACTTCTACATCATCTACATGACCATCC TGCTGCTCCACCGAATCCAGCGAGATCACACCCGATGCAAGGGCAAGTACGGAAAGTACTGGGACGA GTACATGAAGGCCGTGCCTTACAAGCTGATCCCCTACGTGTACTAA
Glycine soja delta-7 sterol reductase	ATGGGCGCTACCGTGCACTCTCCCCTGGTGACCTACGCCTCTGTGATCTCTCTGCTGACCCTGTGTCCT CCTTTCGTGGTGCTGCTGTGGTACACCATGACTCTGGCCGACGGCTCTGTGTCTGAGACTTTCCACTAC CTGCGACAGAACGGCCTGCAGGGCCTGCTGCACATCTGGCCCACTCCTACTCCTACCGCCTGCAAGAT CATTGCCGTGTACGCCGCCTTCGAGGCCGCTCTGCAGCTGCTGCTGCCCGGCAAGACCGTGTACGGCC CCATCTCTCCCACCGGCCACCGACCTGTGTACAAGGCCAACGGACTGCAGGCCTACTTCGTGACCCTG ATCACCTACTTCGCCCTGTGGTGGTTCGGCATCTTCAACCCCACCATCGTGTACCACCACCTGGGCGA GATCTACTCTGCCCTGATCTTCGGCTCTTTCCTGTTCTGCGTGTTCCTGTACATCAAGGGCCATCTGGC TCCCTCTTCTACCGACTCTGGATCTTCTGGCAACCTGATCATCGACTTCTACTGGGGCATGGAACTGTA CCCTCGAATCGGCAAGCACTTCGACATCAAGGTGTTCACCAACTGTCGATTCGGCATGATGTCTTGGG CCGTGCTGGCCCTGACCTACTGCATCAAGCAGTACGAAGAGAACGGCAAGGTGGCCGACTCTATGCT GGTCAACACCGCTCTGATGCTGGTGTACGTGACCAAGTTCTTCTGGTGGGAGGCCGGCTACTGGTCTA CCATGGACATTGCCCACGACCGAGCCGGCTTCTACATCTGCTGGGGCTGCCTGGTGTGGGTGCCCTCT GTGTACACCTCTCCTGGCATGTACCTGGTGAACCATCCTGTGAACCTGGGCATCAAGCTGGCTCTGTC TATCCTGGTGGCCGGCATCCTGTGCATCTACATCAACTACGACTGCGACCGACAGCGACAAGAGTTCC GACGAACTAACGGAAAGGGCACCGTGTGGGGCAAGGCCCCTTCTAAGATCGAGGCCACCTACACCAC TACCTCTGGCGAGACTAAGCGATCCCTGCTGCTGACCTCTGGCTGGTGGGGCCTGTCTCGACACTTCC ACTACGTGCCCGAGATCCTGGCCGCCTTCTTCTGGACCGTGCCTGCTCTGTTCGAGCACTTCCTGCCTT ACTTCTACGTGATCTTCCTGACCATCCTGCTGTTCGACCGAGCTAAGCGAGATGACGACCGATGCCGA TCTAAGTACGGCAAGTACTGGAAGCTGTACTGCGACAAGGTGCCCTACCGAATCATCCCCGGCATCT ACTAA
Tetraselmis sp. GSL018 delta-7 sterol reductase	ATGAAGCGAGCCTCCAAGACCCCCGACACCGCCTCTAAGGGTCGAGAACCCCTTTCTGAGCCTCACA CCAACGGTGTCGCTAAGGCCAGCAACAAGACCTCTTGGGCCGAGTCCAATGGCATCGGTGATCGAGA CGGATTCATGGGCCTGTCCGGTGCCGCCGGCCATGCTGTGGCCCTTCTGGGCACCGTCGTGCTGCTCG TCGGTTGCCCCGCCTTTGTCTTCGTGCTCTGGTACATTAATTGTCGACTCGACGGCTCCGTCTCCGAGT TCGTCGCCCTCGCCGCTCGAGAGGGCGCCGTGGGTCTCTGGCAACGATGGCCTACTCCCACTGCTGAG GCCTGGGCCATCATTGGCACCTTCGGTGCAGTCGAGGCCTTCCTGCAGCTGGCTCTCCCTGGCAAGAA GTTCCTGGGTCCCGTCTCTCCTAAGGGTAACGTCCCCGTCTACAAGGCCAACGGCATGCAAGCCTACG TGACTACCCTGGTCCTGTTCTTTGCCGTCTGGGGCTCCGGCATCTACAACCCCGCGCGAGTCTACGAT CTCATGGGTGAGATTCTGGCCGCCCTGAACATCTTTTCTCTGCTGTTTTGCCTGTTCCTCAACATTAAG GGTCATGTCGCCCCTTCCTCTACTGACTCCGGCTCTACCGGCTCTTTGCTGTACGACTACTACTGGGGC ATGGAGCTTTACCCTAGAATTGGCCGATCCTTCGACATTAAGACCTGGACCAACTGCCGAGTCGGCAT GATGGGTTGGGGCATCCTGATCCTGTGCTACGCCGCCAAGCAGGTGGAGGAGGCTGGATTTCTGTCC GACTCCATGGCTGTGTCCGTCATTCTCATGCACGTTTACATCGCCAAGTTCTTCTGGTGGGAGACTGG TTACTGGAAGACCATGGACATCATGCACGATCGAGCCGGTTACTACATCTGCTGGGGTTGCCTCGTGT GGATCCCCTCCATGTACACCTCTCCCACCATGTTTCTTGTCAAGCATCCTATGGTGCTGGGCCCCACCC TCACCGGCGCTGTCCTGGCTGCTGGCCTGCTGTGTATCTACATCAACTACGACGCCGACCGACAGCGA CAGGTTTTCCGAGAGTCTAACGGCAAGGCCCTGATTTGGGGTCGAAAGCCTAAGAAGATCGAGGCCC AGTACACTACCGCGGACGGCCAAACTAAGACCTCTCTGCTGCTGGTGTCTGGTTGGTGGGGTGTCTCC CGACATTTCCACTACCTGCCCGAGATACTTGCCTCCGTGTTCTGGTCTGTGCCCGCCCAGACTGATTAC GCTATGGCCTACCTGTACTCTGCCTACCTCACCATTCTTCTCGTGGACCGTGCCTTCCGAGACGACCTG

	CGATGCGCTTCCAAGTACGGCAAGCACTGGGTCGAGTATTGCCGACAGGTCCCCCACAAGATCGTGC CCTACATCTTCTAA
Waddlia chondrophila delta-7 sterol reductase	ATGGCCGCCACCACCACCAACGTGCAGACCCGAAACTGGGGCCGAGCCTGGGAGACTACCTGGCTGT CTCTGTTCTCTACCATTGCTCTGCTGGCCACCGCTCCTATGATGGTGCTGTACTGCTACATTGCCTGCG TGCGATTCCGAGGCTCTCTGATCGGACCCGCCTACGCTCTGGCCTCTGGCGCCGTGTCTCTGGACTCT CTGTTCCCCTCGTTCGAGGTGGGCATCTTCGCCCTGTACCTCGGCTGGTTCGCCTTCCAGCTGCTGCTG TACCTGGGACTGCCCGACCTGCTGCACCGAATTCTGCCCCGATACCGAGGCGGCCGACAAGAGGGCG CTGTGACCCCTGCCGGCAAGCAGCTGGTGTACCAGATCAACGGACTGCAGGCCTGGCTGATCTCCCA CCTGTTCTTCGGCATCGGCGCCTACGTGCTCGGATGGTTCTCTCCCTCGATCATTGCCGAGAACTGGG GAGGCTTCCTGATCGTGACCAACGTGATGGGCTACCTGACCGCCATCTTCGTGTACGTGAAGGCCTAC CGATTTCCTTCGAACGCCGAGGACCGAAAGTTCTCTGGCAACCCTCTGTACGACTTCTTCATGGGCAT CGAGTTCAACCCTCGAATCGGCAAGTTCGACTTCAAGCTGTTCTTCAACGGACGACCCGGCATCATTG CTTGGACCCTGATCAACTGGTCTTTCGCCGCCAAGCAGTACGCCGACCTGGGATACCTGCCTAACTCT ATGCTGCTGGTGAACGTGCTGCAGGCTATCTACGTGCTGGACTTCTTCTGGCACGAGACTTGGTATCT CAAGACCATCGACATCTGCCACGACCACTTCGGCTGGATGCTGTCTTGGGGCGACCTGGTGTGGCTGC CCTACATGTACACCCTCCAGGGCCTGTACCTGCTCTACCATCCTGTGGACCTGTCTACCGGCTTCGCTC TGTTCGTGCTGACCCTGGGCGTCGTGGGCTACGCCATTTTCCGATCTGCCAACCACCAGAAGGACCAC TTCCGACGAGTGCAAGGCAAGGAACCCATCTGGGGAAAGATGCCCGAGTTCATCTCTTGCCAGTATA CCGCCGCTGACGGCTCTCTGCACCACACCAAGCTGCTCCTCTCCGGCTGGTGGGGACGAGCCCGACA CATGAACTACACCGGCGACCTGATGCTGTCCCTGGCCTACTGCCTGGCCTGCGGCTTCTCTCACCTCC TGCCTTACTTCTACTTCGTCTACATGACCATCCTGCTGGTCAACCGATGCTACCGAGATGAGCACCGA TGCGAGAACAAGTACGGCGACGCCTGGCGAAAGTACTGCCGACGAGTCCCCTACCGACTGATCCCCG GCATCTACTAA
Tetrahymena thermophilia squalene-tetrahymanol cyclase	ATGAAGAAGATCCTCATCGGTCTCATCATCGGTCTCTTCCTCTTCTCCTCCGTCAACGCCTCCGTCAAC CTCACCGAGGTCCAGAACGCCATCTCCATCCAGCAGGGTATCAACTGGGCTGAGGTCCACAACAACA CCTGGTACTACCCTCCCTACCTCGGTGAGATGTTCATCTCCGAGTACTACTTCGAGCTCCTCGTCCTCA ACTGGACCCACAAGTCCGCCTTCAACGCCACCTACTTCACCGAGCGACTCCTCCAGACCCAGTTCGAG GACGGTTCCTGGGAGCAGGTCCGAGAGCAGAACCTCGAGACCGGTCAGCTCGACGCCACCGTCTTCA ACTACTGGTACCTCAAGTCCATCAACAACAACCCCAAGATCGAGGCTGCCCTCCAGAAGGCCCGAAA GTGGATCGTCGCTCAGGGTGGTATCGAGGCCACCCAGACCATGACCAAGTTCAAGCTCGCTGCCTTC GGTCAGTACTCCTGGGAGGACCTCTGGTACGTCCCTCTCTTCATCTTCAAGCAGAACGGTATCTTCAA GTACACCTACGTCAAGGACATCGTCGCCCAGTGGGTCTACCCCCACCTCACCGCTCTCGCCTACCTCC GATACCAGCGAACCGTCTTCAACGTCCCTGTCGCTGACCTCCGAGAGCTCTGGATCAACTACCCCAAG AACGGTATCAAGATCTCCCCCCGAGAGTACTCCACCCTCAACCCCGACTCCGACCTCCTCATCCTCAT GGACGAGATCTTCAAGCTCAAGCAGCCTCTCGGTTCCTTCGGTGCCTACACCATCTCCACCCTCCTCA CCCTCATGTCCTTCAAGGACTTCCAGTCCAAGCACCCCCACCTCTACCAGAACGAGATCCAGAAGGCC TACGAGGACGGTTACTACTTCGTCGAGTTCAACTACTTCAACTTCCGAGAGGCCTACCACGGTTCCCT CGACGACGGTCGATGGTGGGACACCATCCTCATCTCCTGGGCCATGCTCGAGTCCGGTCAGGACAAG GAGCGAATCTTCCCCATCGTCCAGAACATGGTCAAGGAGGGTCTCCAGCCCAAGAAGGGTATCGGTT ACGGTTACGACTTCGAGTACGCTCCCGACACCGACGACACCGGTCTCCTTCTCGTCGTCATGTCCTAC TACAAGGAGGCCTTCCAGAAGCAGATCCCCGAGACCATCGAGTGGCTCTTCTCCATGCAGAACGACG ACGGTGGTTACCCCGCCTTCGACAAGGGTAAGAACGAGGACAACCTCCTCTTCAAGTTCGCCTTCAAC ATGGCCGGTATCGCCAACTCCGCCGAGATCTTCGACCCTTCCTGCCCTGACATCACCGGTCACATCAT GGAGGGTCTCGGTGAGTTCGGTTACCAGGCCAACCACCCCCAGATCCAGAACATGATCAAGTACCAG CGAAAGACCCAGAACAAGTGGGGTTCCTGGCAGGCTCGATGGGGTGTCAACTACATCATGGCTGTCG GTGCTGTCGTTCCTGGTCTCGCTCGAGTCAACTACGACCTCAACGAGCAGTGGGTCCAGAACTCCATC AACTACCTCCTCAACAAGCAGAACAAGGATGGCGGCTTCGGTGAGTGCGTCCTCTCCTACAACGACC CCGAGAAGTGGAACGGTATCGGTAAGTCCACCGTCACCCAGACCTCCTGGGGTCTCCTCGCTCTCCTC GAGGTCTACAACCAGAACGAGCAGATCAAGCACGCTGCCGATCGAGCTGCCCAGTACCTCCTCGACC AGTTCAAGCGAGACGACAACACCTTCTACGACCACTCCACCATCGGTACCGGTCACCGAGGTCTCCTC TACCTCCAGTACCCCTCCTACGCCCAGTCCTTCCCTCTCGTCGCCCTCAACCGATACCAGAAGATCTCC CAGGGTCAGTACCACTTCTCCAAGAACCTCTACAACGGTAACGGTGAGCCCGTCCAGAAGCAGAACA TCTAA
Solanum lycopersicum delta-24(25) sterol reductase	ATGTCTGACGCCAAGGCTCCCGTGGCCACTGCTTACCCCAAGCGAAAGATCCAGCTGGTGGACTTCCT GCTGTCTTTCCGATGGATCATCGTGATTTTCTTCGTGCTGCCCTTCTCTTTCCTGTACTACTTCTCTATC TACCTGGGCGACGTGAAGTCTGAGCGAAAGTCTTACAAGCAGCGACAGATGGAACACGACGAGAAC GTGAAGGAAGTGGTGAAGCGACTGGGCCAGCGAAACGCCGAGAAGGACGGCCTGGTGTGCACCGCT CGACCTCCATGGGTCGTCGTGGGCATGCGAAACGTGGACTACAAGCGAGCCCGACACTTCGAGGTGG ACCTGTCTAAGTTCCGAAACATCCTGGACATCGACACCGAGCGAATGGTGGCCAAGGTCGAGCCCCT GGTGAACATGGGCCAGATGTCTCGAGTGACCATTCCTATGAACCTGTCTCTGGCCGTGCTGGCCGAGC TGGACGACCTGACCGTCGGCGGCCTGATCAACGGCTTCGGCGTCGAGGGATCTTCTCACATCTTCGGC CTGTTCTCTGACACCGTGGTGGCCCTCGAGGTGGTGCTGGCTGACGGCAAGGTGGTGCGAGCCACCA AGGACAACGAGTACTCTGACCTGTTCTACGCTATCCCCTGGTCGCAGGGCACCCTGGGCCTGCTGGTG TCTGCCGAGATCAAGCTGATCCCCGTGGACCAGTACGTGAAGCTGACCTACAAGCCCGTGCGAGGCA ACCTGAAGGAACTGGCCCAGGCCTACGCCGACTCTTTCGCTCCCAAGGACGGCGACCAGGACAACCC

	CTCTAAGGTGCCCGAGATGGTCGAGGGCATGATCTACGGCCCCACCGAGGGCGTGATGATGACCGGC ATGTACGCCTCTCGAAACGAGGCCAAGCGACGAGGCAACGTGATCAACAACTACGGCTGGTGGTTCA AGCCCTGGTTCTACCAGCACGCTCAGACCGCTCTGAAGCGAGGCGAGTTCGTCGAGTACATCCCTACT CGAGACTACTACCACCGACACACCCGATCTCTGTACTGGGAGGGCAAGCTGATTCTGCCCTTCGGTGA CCAGTTCTGGTTCCGATTCCTGCTCGGCTGGCTGATGCCTCCTAAGATCGCCCTGCTGAAGGCTACCC AGTCTGAGGCCATCCGAAACTACTATCACGACCACCACGTGATCCAGGACCTGCTCGTGCCCCTGTAC AAGGTGGGCGACTGCCTCGAGTGGGTGCACCGAGAGATGGAAGTGTACCCCATCTGGCTGTGTCCCC ACCGAATCTACAAGCTGCCTGTGCGACCTATGATCTACCCCGAGCCTGGCTTCGAGAAGCACAAGCG ACAGGGTGACACCGAGTACGCCCAGATGTACACCGACGTGGGCGTGTACTACGTGCCCGGTGCCGTG CTGCGAGGTGAGCCCTTCGACGGCTCTGAGAAGTGCCGACAGCTGGAACTGTGGCTGATCGAGAACC ACGGCTTCCAGGCTCAGTACGCCGTGACCGAGCTGACCGAGAAGAACTTCTGGCGAATGTTCGACAA CGGCCTGTACGAGCAGTGCCGACGAAAGTACAAGGCCATCGGCACCTTCATGTCTGTGTACTACAAG TCTAAGAAGGGCCGAAAGACTGAGAAGGAAGTTCAAGAGGCCGAGCAAGAGAAGGCTGAGCAAGA AACCCCTGAGGCCAACTAA
Chenopodium quinoa sterol methyl transferase	ATGGACTCTATGGCCCTGATCTGCACCGTGGGCCTGCTGTTCGGCGGCCTGTACTGGTTCATCTGCAT TCACGGACCCGCCGAGCGAAAGGGCAAGCGAGCCGTGGACCTGTCTGGCGGCTCTATCTCTTCTGAC AAGGTGCAGGACAAGTACCAGCAGTACTGGTCGTTCTTCCGACGACCTAAGGAAATCGAGACTGCCG AGAAGGTGCCCGACTTCGTGGACACCTTCTACAACCTGGTGACCGACATCTACGAGTGGGGCTGGGG CCAGTCTTTCCACTTCTCTCCCTCGATTCCCGGCAAGTCTCACCGAGATGCTACCCGAATCCACGAAG AGATGGCCGTCGACCTGATCAAGGTGTCTCCCGGCCAAAAGATCCTGGACGTCGGCTGCGGCGTCGG CGGACCCATGCGAGCCATTGCCGCTCACTCTCGAGCCAAGGTGACCGGCATCACCATCAACGAGTAC CAGGTGAAGCGAGCCAAGCTGCACAACAAGAAGGCCGGACTGGACTCTCTGTGCGAGGTGGTGTGC GGCAACTTCCTCGAGATGCCCTTCGCCTCTAACACCTTCGACGGCGCCTACTCTATCGAGGCCACCTG TCACGCTCCCAAGCTGGACGACGTGTACTCTGAGATCTTCCGAGTGCTGAAGCCCGGCTCTCTGTACG TGTCTTACGAATGGGTGACCACCGACAAGTTCAACGGCGACGACTCTGAGCACTGCGACGTGATCCA GGGCATCGAGCGAGGCGACGCTCTGCCCGGCCTGCGACGATACGACGAGATCTCTGAGGCCGCCAAG AAGGTGGGCTTCGAGATCGTGGACGAGCGAGATCTGGCCGCTCCTCCTGCTAAGCCCTGGTGGGACC GACTGAAGATGGGCCGAATCGCCTACTGGCGAAACCACATCGTGGTGACCGTGCTGGCCGCCATCGG CGTGGCTCCCAAGGGCACCGTGGACGTGCACGACATGCTGTTCAAGACCGCCGACTACCTGACTCGA GGCGGCGAGTCTGGCATTTTCTCTCCCATGCACATGATCCTGTGTCGAAAGCCCGTGGACGCCAAGTC TGACTCTTAA
Solanum tuberosum delta-24(28) sterol reductase	ATGACCGACGTCCAGGCCCCTCCTCGACCCAAGCGAAAGAAGAACATCATGGACCTCCTCGTCCAGT TCCGATGGATCGTCGTCATCTTCGTCGTCCTCCCCCTCTCCTTCCTCTACTACTTCTCCATCTACGTCGG TGACGTCCGATCCGAGTGCAAGTCCTACAAGCAGCGACAGAAGGAGCACGACGAGAACGTCAAGAA GGTCGTCAAGCGACTCAAGGACCGAAACGCCTCCAAGGACGGTCTGGTCTGCACCGCCCGAAAGCCC TGGGTCGCTGTCGGTATGCGAAACGTCGACTACAAGCGAGCCCGACACTTCGAGGTCGACCTCTCCC CCTTCCGAAACGTCCTCAACATCGACACCGAGCGAATGATCGCCAAGGTCGAGCCCCTCGTCAACAT GGGTCAGATCTCCCGAGTCACCGTCCCCATGAACGTCTCCCTCGCCGTCGTCGCTGAGCTCGACGACC TCACCGTCGGTGGTCTGATCAACGGTTACGGTATCGAGGGTTCCTCCCACATCTACGGTCTGTTCTCC GACACCGTCGTCTCCTACGAGGTCGTCCTCGCCGACGGTCAGGTCGTCCGAGCCACCAAGGACAACG AGTACTCCGACCTCTTCTACGCCATCCCCTGGTCCCAGGGTACCCTCGGTCTGCTCGTCTCCGCCGAG ATCAAGCTCATCCCCATCAAGGAGTACATGAAGCTCACCTACAAGCCCGTCGTCGGTAACCTCAAGG AGATCGCCCAGGCCTACATCGACTCCTTCTCCCCCAAGGACGGTGACCAGGACAACCGAGAGAAGGT CCCCGACTTCGTCGAGACTATGGTCTACACCCCCACCGAGGCTGTCTGCATGACCGGTCGATACGCCT CCAAGGAGGAGGCCAAGAAGAAGGGTAACGTCATCAACAACGTCGGTTGGTGGTTCAAGACCTGGTT CTACCAGCACGCCCAGACCGCTCTCAAGAAGGGTGAGTTCGTCGAGTACATCCCCACCCGAGAGTAC TACCACCGACACACCCGATGCCTCTACTGGGAGGGTAAGCTCATCCTCCCCTTCGGTGACCAGTGGTG GTTCCGATTCTTCTTCGGTTGGGCCATGCCCCCTAAGGTTTCCCTCCTCAAGGCCACCCAGGGTGAGT ACATCCGAAACTACTACCACGAGAACCACGTCATCCAGGACATGCTCGTCCCCCTCTACAAGGTCGG TGACGCCCTCGAGTGGGTCAACCGAGAGATGGAGGTCTACCCCCTCTGGCTCTGCCCCCACCGACTCT ACCGACTCCCCCTCAAGACCATGGTCTACCCCGAGCCTGGTTTCGAGCTCCACAAGCGACAGGGTGA CACCAAGTACGCCCAGATGTACACCGACGTCGGTGTCTACTACGCCCCCGGTCCCATCCTCCGAGGTG AGGTCTTCGACGGTATCGAGGCCGTCCGAAAGCTCGAGTCCTGGCTCATCGAGAACCACGGTTTCCA GCCCCAGTACGCCGTCTCCGAGCTCACCGAGAAGAACTTCTGGCGAATGTTCGACGGTTCCCTCTACG AGAACTGCCGAAAGAAGTACCGAGCCATCGGTACCTTCATGTCCGTCTACTACAAGTCCAAGAAGGG TAAGAAGACCGAGAAGGAGGTCCAGGACGCCGAGCAGGAGACTGCCGAGGTCGAGACTCCCGAGGT CGACGAGCCCGAGGACTAA

Supplementary Table 4.

Plasmids used for strain engineering in this study. The corresponding parental plasmids and BioBricks that were used to construct each plasmid are given.

Plasmid name	Parent plasmid/ Reference	BioBricks
pCfB6611 (pNat-PrExp-Cre)	pCfB4158 (pPrExp-Cre), Holkenbrink *et al,* 2018 [31]	pCfB4158 backbone: BB1037(Fragment1EpiVecYL)
pCfB6633 (pNat-YLgRNA2_IntE_1)	Holkenbrink *et al,* 2018[31]	
pCfB6637 (pNat-YLgRNA3_IntE_3)	Holkenbrink *et al,* 2018[31]	
pCfB6638 (pNat-YLgRNA2_IntE_4)	Holkenbrink *et al,* 2018[31]	
pCfB6677 (pIntE_1-TPex20-TLip2)	Holkenbrink *et al,* 2018[31]	
pCfB6679 (pIntE_4-TPex20-TLip2)	Holkenbrink *et al,* 2018[31]	
pCfB6681 (pIntE_3-TPex20-TLip2)	Holkenbrink *et al,* 2018[31]	
pBP8003 (pNat-YLgRNA4-IntF_3)	Dr. K.R. Kildegaard, Biophero ApS, Denmark	
pBP8009 (pIntF_3-TPex20-TLip2)	Dr. K.R. Kildegaard, Biophero ApS, Denmark	
pBP8660 (pIntE_5-TPex20-TLip2)	Dr. K.R. Kildegaard, Biophero ApS, Denmark	
pCfB8861 (pHyg-YLgRNA3_IntE_4)	Arnesen *et al,* 2020[23]	
pCfB10249 (IntE4_PrTEFInt->DHCR7_Stuberosum)	pCfB6679	BB3879 (TefInt->):BB4876 (PrTEFInt_DHCR7St)
pCfB10250 (IntE4_PrTEFInt->DHCR7_Drerio)	pCfB6679	BB3879 (TefInt->):BB4877 (PrTEFInt_DHCR7Dr)
pCfB10251 (IntE4_PrTEFInt->DHCR7_Ldrancou)	pCfB6679	BB3879 (TefInt->):BB4878 (PrTEFInt_DHCR7Ld)
pCfB10252 (IntE4_PrTEFInt->DHCR7_Esilicul)	pCfB6679	BB3879 (TefInt->):BB4879 (PrTEFInt_DHCR7Es)
pCfB10253 (IntE4_PrTEFInt->DHCR7_Cprotoch)	pCfB6679	BB3879 (TefInt->):BB4880 (PrTEFInt_DHCR7Cp)
pCfB10254 (IntE4_PrTEFInt->DHCR7_Csubellip)	pCfB6679	BB3879 (TefInt->):BB4881 (PrTEFInt_DHCR7Cs)
pCfB10255 (IntE4_PrTEFInt->DHCR7_Mverticillata)	pCfB6679	BB3879 (TefInt->):BB4882 (PrTEFInt_DHCR7Mv)
pCfB10256 (IntE4_PrTEFInt->DHCR7_Gsoja)	pCfB6679	BB3879 (TefInt->):BB4883 (PrTEFInt_DHCR7Gs)
pCfB10257 (IntE4_PrTEFInt->DHCR7_Tsp)	pCfB6679	BB3879 (TefInt->):BB4884 (PrTEFInt_DHCR7Ts)
pCfB10258 (IntE4_PrTEFInt->DHCR7_Wchondrophi)	pCfB6679	BB3879 (TefInt->):BB4885 (PrTEFInt_DHCR7Wc)
pCfB10494 (IntE3_PrGPAT->TtSTC)	pCfB6681	BB1617 (PrGPAT): BB5100 (TtSTC_USER)
pCfB10783(gRNA_IntE_5)	Dr. J. Dahlin, DTU CfB, Denmark	
pCfB10851 (IntE1_PrGPAT->SMT2_Cquinoa)	pCfB6677	BB1617 (PrGPAT->): BB5371 (PrGPAT_SMT2Cq)
pCfB10853 (IntE5_PrGPAT->DHCR24_Slycopersic)	pBP8660	BB1617 (PrGPAT->): BB5373 (PrGPAT_DHCR24Sl)
pCfB10948 (IntF3_PrDGA1->StSSR1)	pCfB8009	BB1616 (PrDGA1): BB5456 (StSSR1_USER)

Supplementary Table 5.
BioBricks used in this study. The DNA template and primers used for the amplification of each BioBrick are given.

BioBrick name	**Template/Reference**	**Forward primer**	**Reverse primer**
pCfB4158 backbone	pCfB4158, Holkenbrink *et al,* 2018[31]	10593	10594
BB1037(Fragment1EpiVecYL)	Holkenbrink *et al,* 2018[31]	10591	10592
BB1616 (PrDGA1->)	Holkenbrink *et al,* 2018[31]		
BB1617 (PrGPAT->)	Holkenbrink *et al,* 2018[31]		
BB3879 (PrTefInt->)	Arnesen *et al,* 2020[23]		
BB4876 (PrTEF_DHCR7St)	*Solanum tuberosum* delta-7 sterol reductase codon optimised	PR-27565	PR-27566
BB4877 (PrTEF_DHCR7Dr)	*Danio rerio* delta-7 sterol reductase codon optimised	PR-27567	PR-27568
BB4878 (PrTEF_DHCR7Ld)	*Legionella drancourtii* delta-7 sterol reductase codon optimised	PR-27569	PR-27560
BB4879 (PrTEF_DHCR7Es)	*Ectocarpus siliculosus* delta-7 sterol reductase codon optimised	PR-27571	PR-27572
BB4880 (PrTEF_DHCR7Cp)	Candidatus *Protochlamydia amoebophila* delta-7 sterol reductase codon optimised	PR-27573	PR-27574
BB4881 (PrTEF_DHCR7Cs)	*Coccomyxa subellipsoidea* delta-7 sterol reductase codon optimised	PR-27575	PR-27576
BB4882 (PrTEF_DHCR7Mv)	*Mortierella verticillate* delta-7 sterol reductase codon optimised	PR-27577	PR-27578
BB4883 (PrTEF_DHCR7Gs)	*Glycine soja* delta-7 sterol reductase codon optimised	PR-27579	PR-27570
BB4884 (PrTEF_DHCR7Ts)	*Tetraselmis* sp. GSL018 delta-7 sterol reductase codon optimised	PR-27581	PR-27582
BB4885 (PrTEF_DHCR7Wc)	*Waddlia chondrophila* delta-7 sterol reductase codon optimised	PR-27583	PR-27584
BB5041 (LoxP_HphMX)	pCfB5935, Holkenbrink *et al,* 2018[31]	PR-27972	PR-27973
BB5091 (*ERG4*_1kbUP_U1)	ST9100 gDNA	PR-27891	PR-28358
BB5092 (*ERG4*_1kbDOWN_U2)	ST9100 gDNA	PR-27893	PR-27894
BB5093 (*ERG5*_1kbUP_U1)	ST9100 gDNA	PR-27895	PR-28359
BB5094 (*ERG5*_1kbDOWN_U2)	ST9100 gDNA	PR-27897	PR-27898
BB5097 (*ERG4*_HphMX_KO)	BB5091 + BB5092 + BB5041 USER reaction	PR-27891	PR-27894
BB5098 (*ERG5*_HphMX_KO)	BB5093 + BB5094 + BB5041 USER reaction	PR-27895	PR-27898
BB5100 (TtSTC_USER)	*Tetrahymena thermophilia* squalene-tetrahymanol cyclase codon optimised	PR-28361	PR-28362
BB5371 (PrGPAT_SMT2Cq)	*Chenopodium quinoa* sterol methyl transferase codon optimised	PR-27621	PR-27786
BB5373 (PrGPAT_DHCR24Sl)	*Solanum lycopersicum* delta-24(25) sterol reductase codon optimised	PR-27599	PR-27764
BB5456 (StSSR1_USER)	*Solanum tuberosum* delta-24(28) sterol reductase codon optimised	PR-29404	PR-29405

Supplementary Table 6.
Primers used in this study.

Primer name	**Sequence (5’ → 3’)**
PR-14617 (vector verification *E. coli* cPCR)	tatccctgtgttgaatc
PR-14619 (vector verification *E. coli* cPCR)	tatcgacccagttagc
PR-8859 (integration verification Yl cPCR)	aagtgtggatggggaagtgag
PR-14442 (IntE_1 verification Yl cPCR)	agttgtgaccaagacaaatg
PR-14398 (IntE_1 verification Yl cPCR)	cacgcgaUgttagaagcaattggagaag
PR-14576 (IntE_3 verification Yl cPCR)	cacgcgautgaaggaaatgcctaaaacc
PR-14835 (IntE_3 verification Yl cPCR)	cacgcacgccattctataag
PR-14592 (IntE_4 verification Yl cPCR)	acgcgauttaacactggaccgtactgc
PR-20880 (IntE_4 verification Yl cPCR)	attgctaagcgaccatagac
PR-14837 (IntF_3 verification Yl cPCR)	acatgctcgcgcctcgatag
PR-14584 (IntF_3 verificiationYl cPCR)	cacgcgautttggtcgtcgcccaacaag
PR-26834 (IntE_5 verification Yl cPCR)	gggacaagtgcaatcgttcacttg
PR-26835 (IntE_5 verification Yl cPCR)	cgttgctgttgccacaatggac
PR-27891 (Erg4Rep_P1F)	ctctcaacaccttcaccgc
PR-28358 (Erg4Rep_P1R_U1)	atcgcacgugataagcttagtgagcgaatgg
PR-27893 (Erg4Rep_P2F)	gtgcaggutgtgtggttgcgaaggaag
PR-27894 (Erg4Rep_P2R)	gcactcaaaataccccgttc
PR-27895 (Erg5Rep_P1F)	ctcggtttgttgcagcagg
PR-28359 (Erg5Rep_P1R_U1)	atcgcacgutggtccgtatcgtgaaatgg
PR-27897 (Erg5Rep_P2F)	gtgcaggugggcggagttgtgttgtg
PR-27898 (Erg5Rep_P2R)	ggtcggctatccaatacatctc
PR-27972 (HphMX_F_U1)	cgtgcgautcagctgaagcttcgtac
PR-27973 (HphMX_R_U2)	acctgcacugcataggccactagtgg
PR-28361 (TtSTC_F_U3)	atctgtcaugccacaatgaagaagatcctcatcggtc
PR-28362 (TtSTC_Rv)	cacgcgauttagatgttctgcttctggacg
PR-22830 (erg5Δ_chk)	tcatactcaccgaaacgtg
PR-27961 (erg5Δ_chk)	gttccaatgcctggcaag
PR-27634 (erg5Δ_chk)	acttctctctcacaccacc
PR-27635 (erg5Δ_chk)	ctgagggctctgttggtgaag
PR-27636 (erg5Δ_chk)	accagtgtggttgtaaggatg
PR-11138 (erg4Δ_chk)	agcaatggguaaaaagcctgaactcaccgc
PR-27631 (erg4Δ_chk)	cctgatattggtgatcctcc
PR-27632 (erg4Δ_chk)	agagccttgtttccgaggtg
PR-27633 (erg4Δ_chk)	atacaatcccataggctggc
PR-28001 (erg4Δ_chk)	cgtgcgaugcttgccctggactacatcttg
PR-27565 (A_St_Fw)	actttttgcagtacuaaccgcaggccgagtctcagctggtgcac
PR-27566 (A_St_Rv)	cacgcgauttagtagatgccggggatcac
PR-27567 (A_Dr_Fw)	actttttgcagtacuaaccgcagatggcctctgaccgagttcg
PR-27568 (A_Dr_Rv)	cacgcgauttagaagatgttgggcagcag
PR-27569 (A_Ld_Fw)	actttttgcagtacuaaccgcagtacttcaagatccgaaacac
PR-27570 (A_Ld_Rv)	cacgcgauttagatcacgaagggcacgatc
PR-27571 (A_Es_Fw)	actttttgcagtacuaaccgcagatcgacggcgctgccatcg
PR-27572 (A_Es_Rv)	cacgcgauttacaggatgccgggcacgatc
PR-27573 (A_Cp_Fw)	actttttgcagtacuaaccgcagctgatcgagatgctgtgcatc
PR-27574 (A_Cp_Rv)	cacgcgauttagatcacgaaggggatgatc
PR-27575 (A_Cs_Fw)	actttttgcagtacuaaccgcaggtcacaacccgagccgctg
PR-27576 (A_Cs_Rv)	cacgcgauttagaaaatgtaagggatcatc
PR-27577 (A_Mv_Fw)	actttttgcagtacuaaccgcaggccgtgcagcagcgaaagac
PR-27578 (A_Mv_Rv)	cacgcgauttagtacacgtaggggatcagc
PR-27579 (A_Gs_Fw)	actttttgcagtacuaaccgcagggcgctaccgtgcactctc
PR-27580 (A_Gs_Rv)	cacgcgauttagtagatgccggggatg
PR-27581 (A_Ts_Fw)	actttttgcagtacuaaccgcagaagcgagcctccaagaccc
PR-27582 (A_Ts_Rv)	cacgcgauttagaagatgtagggcacgatc
PR-27583 (A_Wc_Fw)	actttttgcagtacuaaccgcaggccgccaccaccaccaacg
PR-27584 (A_Wc_Rv)	cacgcgauttagtagatgccggggatcag

PR-27599 (B_Sl_Fw)	atctgtcaugccacaatgtctgacgccaaggctc
PR-27764 (B_Sl_Rv)	cacgcgauttagttggcctcagggg
PR-27621 (C_Cq_Fw)	atctgtcaugccacaatggactctatggccctg
PR-27786 (C_Cq_Rv)	cacgcgauttaagagtcagacttggc
PR-29404 (StSSR1_F_U3)	atctgtcaugccacaatgaccgacgtccagg
PR-29405 (StSSR1_Rv)	cacgcgauttagtcctcgggctcgtc

Supplementary Table 7.

Sterol composition of diets prepared for feeding trials (values given to 3 d.p. ± standard deviation).

	Dietary sterol content (w/w)				Percentage of total sterol (%)			
Sterol	**MxSt**	**WT**	**Tet**	**Base**	**MxSt**	**WT**	**Tet**	**Base**
24-Methylenecholesterol	0.146 ± 0.040	0	0	0	43.3 ± 4.15	0.300 ± 0.068	0.228 ± 0.037	0.131 ± 0.051
Campesterol	0.038 ± 0.028	0.016 ± 0.000	0.015 ± 0.002	0.069 ± 0.011	13.5 ± 10.8	14.4 ± 1.28	11.3 ± 0.647	20.1 ± 1.45
Cholesterol	0.008 ± 0.000	0	0	0.001 ± 0.000	2.61 ± 0.496	0.421 ± 0.073	0.257 ± 0.022	0.209 ± 0.015
Desmosterol	0	0	0	0	0.114 ± 0.037	0	0	0
Isofucosterol	0.014 ± 0.005	0.016 ± 0.001	0.014 ± 0.001	0.053 ± 0.011	4.6 ± 2.38	14.2 ± 0.267	10.3 ± 0.876	15.9 ± 4.28
Sitosterol	0.022 ± 0.004	0.026 ± 0.002	0.023 ± 0.002	0.073 ± 0.004	7.06 ± 2.60	23.5 ± 1.42	16.6 ± 1.54	21.6 ± 2.45
Tetrahymanol	0.073 ± 0.054	0	0.037 ± 0.006	0	19.6 ± 13.3	0	27.1 ± 3.41	0
Zymosterol	0.001 ± 0.000	0.001 ± 0.000	0	0	0.220 ± 0.175	0.574 ± 0.118	0.200 ± 0.021	0
Ergosterol	0	0.015 ± 0.003	0.013 ± 0.001	0	0	13.3 ± 2.31	9.3 ± 0.427	0
Campestanol	0.002 ± 0.002	0.007 ± 0.000	0.007 ± 0.001	0.038 ± 0.008	0.422 ± 0.597	6.26 ± 0.168	5.1 ± 0.643	11.2 ± 2.15
Cycloartenol	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.009 ± 0.001	1.15 ± 0.308	2.56 ± 0.421	2.04 ± 0.119	2.77 ± 0.398
Stigmasterol	0.005 ± 0.001	0.004 ± 0.000	0.003 ± 0.000	0.015 ± 0.003	1.53 ± 0.119	3.24 ± 0.294	2.36 ± 0.241	4.38 ± 0.501
31-Norcycloartanol	0.005 ± 0.000	0.004 ± 0.001	0.004 ± 0.000	0.014 ± 0.002	1.47 ± 0.216	4.02 ± 0.261	3.25 ± 0.172	4.21 ± 0.484
Sitostanol	0.007 ± 0.002	0.013 ± 0.002	0.010 ± 0.001	0.044 ± 0.034	2.17 ± 0.390	11.3 ± 0.794	7.61 ± 1.33	11.8 ± 8.11
24(28)-Methylenecycloartanol	0.007 ± 0.000	0.007 ± 0.001	0.006 ± 0.001	0.026 ± 0.005	2.22 ± 0.582	5.93 ± 0.453	4.35 ± 0.127	7.69 ± 2.10
Total	0.331 ± 0.061	0.111 ± 0.007	0.136 ± 0.010	0.341 ± 0.045	100	100	100	100

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