Supplementary material for the research article

Biosynthesis of insect sex pheromone precursors via engineered β -oxidation in yeast

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Supplementary table S1. Primers used in this study

ID	Description	Sequence 5' to 3'
PR-18928	PrTEF1intron <u1_fw< td=""><td>CACGCGAU AGAGACCGGGTTGG</td></u1_fw<>	CACGCGAU AGAGACCGGGTTGG
PR-18975	<-PrTEF1_fw	ACCTGCACUTTTGAATGATTCTTATAC
PR-18066	Har_FAR_U2_fw	ATCTGTCAUGCCACAATGGTGGTCCTGACCTCTAAG
PR-16595	Har_FAR_codoptYL_U2_rev	CACGCGAUCTACTCGTAGGACTTCTTCTC
PR-18930	PrTEF1intron <- _forfusion_U1_fw	ATCAGTAGCU AGAGACCGGGTTGG
PR-18214	PrTEF1intron_USER_rv	AGTACTGCAAAAAGUGCTG
PR-23004	PrGPD_forfusion_U1_fw	ATCAGTAGCUGACGCAGTAGGATGTCCTGC
PR-22213	PrGAPHD->_U2_rev	ATGACAGAU TGTTGATGTGTGTTTAATTCAAGAATG
PR-19018	Dmd9_U1_rev	CGTGCGAUTTATCGAGACTTGTCC
PR-21723	<-Lbo_PPTQ_U1_fw	ACTTTTTGCAGTACUAACCGCAG GTGCCTCGAGCCGCTTCGG
PR-21724	<-Lbo_PPTQ_U1_rev	CGTGCGAU TTACTCCTTCTTAGCGTG
PR-23435	Ase_POX_U1_fw	AGTGCAGGUGCCACA ATGCCCATCTTCATCTG
PR-23436	Ase_POX_U1_rv	CGTGCGAUTTACAGCTTAGACTGCA
PR-21755	Lbo31670->_U2_fw	ATCTGTCAU GCCACA ATGACTGAGGTGACCAAGG
PR-21756	Lbo31670->_U2_rev	CACGCGAU TTACAGCTTACCCTGCATG
PR-15521	PrExp_fw	CGTGCGAUAAGGAGTTTGGCGCCCGTT
PR-15522	PrExp_rev	ATGACAGAUTGCTGTAGATATGTCTTGT
PR-21757	Lbo49554->_U2_fw	ATCTGTCAU GCCACA ATGGAGTCTAAGGACTTTG
PR-21758	Lbo49554->_U2_rev	CACGCGAUTTACAGCTTGGCGGGCACG
PR-22827	Yli_POX2_fw	AGTGCAGGUGCCACAATGAACCCC
PR-22828	Yli_POX2_rv	CGTGCGAUCTATTCCTCATCAAG
PR-22829	Yli_POX3_fw	AGTGCAGGUGCCACAATGATCTCCC
PR-22830	Yli_POX3_rv	CGTGCGAUCTATTCCTCGTCCAG

PR-22833	Yli_POX5_fw	AGTGCAGGUGCCACAATGAACAACAAC
PR-22834	Yli_POX5_rv	CGTGCGAUCTACTCGTCCAGGTC
PR-22841	Ani_POX_fw	AGTGCAGGUGCCACAATGCCCAACC
PR-22842	Ani_POX_rv	CGTGCGAUCTACAGCTTAGACTTG
PR-22843	Cma_POX_fw	AGTGCAGGUGCCACAATGGCCGCTG
PR-22844	Cma_POX_rv	CGTGCGAUCTACAGCTTAGACGAAG
PR-22845	Hsa_POX1-2_fw	AGTGCAGGUGCCACAATGAACCCCG
PR-22846	Hsa_POX1-2_rv	CGTGCGAUCTACAGCTTCGAAGAAG
PR-22847	Pur_POX_fw	AGTGCAGGUGCCACAATGACCGAGG
PR-22848	Pur_POX_rv	CGTGCGAUCTACAGCTTCGAAGAAG
PR-22849	Rno_POX-2_fw	AGTGCAGGUGCCACAATGAACCCCG
PR-22850	Rno_POX-2_rv	CGTGCGAUCTACAGCTTCGAAGAAG
PR-10595	PrTEF1intron_fw	CGTGCGAUAGAGACCGGGTTGG
PR-24919	HarFAR_Per2_U2_rev	CACGCGAUCTAAAGCTTAGACTTGGCCTGAGAAAGCTT AACGGCGGCGGAGCCTCCGCCCTCGTAGGACTTCTTC
PR-23172	IntD2_dwn_fwd	AGTGGCCUGACCAACCTTGTTTGG
PR-23173	IntD2_dwn_rev	CACGCGAUGCATATACGATTTGACTG
PR-23171	IntD2_up_fwd	CGTGCGAUCGTTTCGGAATGTGTC
PR-23170	IntD2_up_rev	AAGCGTTGCACGUTAAGTTGAGAGAGAACGC
PR-23167	IntE_7_up_fwd	CGTGCGAUGAATCTTGGTGCTCAAC
PR-23166	IntE_7_up_rev	AAGCGTTGCACGUGCAATCCGAAGAAGC
PR-23168	IntE_7_dwn_fwd	AGTGGCCUTACTCACATCAGATGGTC
PR-23169	IntE_7_dwn_rev	CACGCGAUGACACATGTGTCTACG
PR-22532	IntF_5_up_fwd	CGTGCGAUGAAGGCTACAACAAGGG
PR-22533	IntF_5_up_rev	AAGCGTTGCACGUTTTTTACATCACGTGCC
PR-22534	IntF_5_down_fwd	AGTGGCCUTGCGCTCACTCGTGATG
PR-22535	IntF_5_down_rev	CACGCGAUCTTGTTTCCCATAGTTTAATG
PR-26919	HarFARforhrGFP_rv	ACCACCCTCGUAGGACTTCTTCTC
PR-26920	hrGFPforHarFAR_fw	ACGAGGGTGGUGGTTCTGTGAGCAAGCAGATC
PR-15506	hrGFP_U2_rev	
PR-26921	hrGFPPer2_rv	CGGCGGAGCCTCCGCCCACCCGTGCAG
PR-24936	POX3 Y.l. for TEF1intron fw	ACTTTTTGCAGTACUAACCGCAGATCTCCCCCAACCTC
PR-26932	YliPOX3formCherry_rv	ACCACCTUCCTCGTCCAGCTC
PR-26926	YliALE1forTEF1intron_fw	ACTTTTTGCAGTACUAACCGCAGGCCTTTCCATGG
PR-26933	YliALE1formCherry_rv	ACCACCCTUGGTCTTGATGG
PR-26934	mCherryforYliPOX3_fw	AAGGTGGUGGTTCTGTGTCTAAGGGCGAAG
PR-26936	mCherry_U2_rv	CACGCGAUCTACTTGTACAGCTC
PR-26935	mCherryforYliALE1_fw	AAGGGTGGUGGTTCTGTGTCTAAGGGCGAAG

Supplementary table S2. Synthetic genes used in this study

ID	Gene	Sequence 5' to 3'
Sequence No. 1	Fatty acyl	ATGGTGGTCCTGACCTCTAAGGAGACTAAGCCCTCCGTGGCCGAGTTCTACGCTGGCAAGTCTGT
	reductase from	CTTCATCACCGGCGGAACCGGTTTCCTGGGCAAGGTCTTCATTGAGAAGCTGCTGTACTCCTGTC
	Helicoverpa	CCGACATCGGCAACATCTACATGCTGATCCGAGAGAAGAAGGAACTGTCTGT
	armigera	AAGCACTTCCTGGACGACCCCCTGTTCACCCGACTGAAGGAGAAGCGACCCGCCGACCTGGAGAA
		GATCGTGCTGATTCCCGGAGACATCACCGCTCCCGACCTGGGTATTACCTCTGAGAACGAGAAGA
		TGCTGATCGAGAAGGTGTCTGTCATCATTCACTCCGCCGCTACCGTCAAGTTCAACGAGCCCCTG

		CCCACCGCCTGGAAGATCAACGTGGAGGGAACCCGAATGATGCTGGCTCTGTCTCGACGAATGAA GCGAATTGAGGTCTTCATCCACATTTCCACCGCCTACACCAACACCAACCGAGAGGTGGTGGACG AGATCCTGTACCCTGCTGCTGCTGACATTGACCAGGTGCACCGATACGTCAAGGACGGTATCTCT GAGGAAGAGACTGAGAAGATTCTGAACGGCCGACCCAACACCTACACCTTCACCAAGGCCGTGAC CGAGCACCTGGTGGCTGAGAACCAGGCTTACGTGCCCACCATCATTGTCCGACCCTCCGTGGTCGC CGCTATCAAGGACGAGCCCATTAAGGGATGGCTGGCCACCATCATTGTCCGACCCTCCGTGGTCGC CGCTATCAAGGACGAGCCCATTAAGGGATGGCTGGCCACCATCATTGTCCGACCCTCCGTGGTCGC CGCTATCAAGGACGAGCCCATTAAGGGATGGCTGGCCACCTTCCAACACTCGTGGACCTGATT CCCGTGGACTACGTCGCCAACCTGGTCATTGCCGCTGGCGCTAAGTCTTCCAAGTCCACCGAGCTG AAGGTGTACAACTGTTGCTCTTCCGCCTGCAACCCGACCCATCACCAAGTCCACCGAGCTG CGCGAGGACGCTATCAAGCAGAAGTCCTACGCTATGCCCCTGCCCGGTTGGTACATCTTACCGAC AGTACAAGTGGCTGGTCCTGCTGACCATTCTGTTCCAGGTCATCCCGCCTACATTACCGACC TGTACCGACACCTGATCGGCAAGAACCCCCGATACATTAAGCTGCAGTCTCTGGTCAACCAGACC CGATCTTCCATTGACTTCTTCACCTCTCACCTGTTCCCCCTGGACACCCAGCCCGAGCCT GTTCGCCTCTCTGTCCCCCGCTGACACTACCTGTTCCCCCGGCCACCCAC
Sequence No. 2	Fatty acyl desaturase from Drosophila melanogaster	ATGGCTCCCTACTCTCGAATCTACCACCAGGACAAGTCGTCCCGAGAGACTGGCGTGCTGTTCGA GGACGACGCCCAGACCGTGGACTCTGACCTGAC
Sequence No. 3	Fatty acyl desaturase from <i>Lobesia</i> <i>botrana</i>	ATGGTGCCTCGAGCCGCTTCGGAGGAGACCGACCTTAAGGAGGCTACCCAGCTTGAGCCCCGAAA GTACGAGATCGTGTACACTAACGTGATCTACTTCACCTATTGGCATATCGCCGGACTGTACGGTC TGTACCTGTGTTTTACCTCCGCTAAATGGGAGACCATCGTGTTCGGTTGGGCTTGGGATGGCGCT GGAGAGCTGGGAGTGATTGCCGGCGCTCATAGATTGTGGGCCCACCGAACCAACAGGAAAGAT GCCCCTGCAGATCATCCTGATGCTGTTTAATTGTATCGGGTTTTCAGAACACCGCTACAGGGAAAGAT GCCCTTGCAGATCACCGAGTGCATCACAAGCACTCTGACACCGGACGCCCCCATAACTCTCAGCGG TTCGAGATCACCGAGTGCATCACAAGCACTCTGACACCGGACGCGCCCCCATAACTCTCAGCGG GGCTTCTTTTTCTCTCACGTGGGCTGGCTGGCTGACCGACAGCGCCGCCCCCATAACTCTCAGCGA GGCTTGTTGACATGACTGATATCTACTCTAC
Sequence No. 4	Peroxisomal oxidase from Agrotis segetum	ATGCCCATCTTCATCTGCATCATCACCTCTCAGGCCATCATCCGATCTAACGTCGAGGCGAGGCGAGGCC GTGATCCTGAACATCAACATGGGCAAGGTGAACGAGGGCCTGGTGCGAGAGCGAGC

		TCTACCTTCTACGAGGACATGTCTAAGGCCATGCGATCTATGACCGCTCCTCTGGCTAAGGTGAT
		GGGCCAGCTGGTCGAGCTGTACGCTGTGTACTGGACTCTCGAGCGACTGGGAGACATGCTGCAGT
		CTGGGCGCCTACGACGGCCGAGTGTACGAGCGACTCATGGAAGAGGCCCTGAAGTCTCCCCTGAA
		CGCTGAGCCCGTGAACCAGTCTTTCCACAAGTACCTGAAGCCTTTCATGCAGTCTAAGCTGTAA
Sequence No. 5	Peroxisomal	ATGACTGAGGTGACCAAGGTGAACCCCGACCTCCAGCGAGAACGAGACAACTGTACATTCAACGT
	oxidase 31760	TACCGAGCTGACTAACCTGATCGACGGCGGTGTGCAGAAGACCGAGGGGGGGG
	from Lobesia	AGATGGTGCTGAAAGAAGGTATCCACCTGGACGAGGTTCCCTCAGAGTACCTGTCCCACAAGGAG
	botrana	AAGTACGAGCTCGCTGTGAAGAAGGCATGCTACCTGTTCAAGATGATCCGACGACTGCAGGAGGA
		TTGAGCAGCAAGTGCTTTTGGATCGGCCGAGCCTTCAATTGCGGAACTTACGGAACTTACGCCAG
		ACTGAGCTTGGTCACGGAACCTTCATTCGAGGACTGGAAACCACCGCCACTTACGATCCCTCTAC
		AAAGGAGTTCGTCCTGCACTCCCCCACTTTGACTTCCTACAAGTGGTGGCCCGGAGGCCTTGCTC
		ACACGGCCAATTACTGTATCGTGGTGGCTCAACTTTACACACAAGGCAAGTGCCATGGCATTCAC
		CCCTTTATCGTCCAGCTACGAGACGAAGAGACCCACATGCCCCTGCCCGGCATCAAGGTGGGCGA
		AATCGGCGCTAAACTGGGAATGAACGGCACCAACAACGGCTTCCTTGGATTCGATAAGGTGCGAA
		CUCAGLAGIAAGCIGACAIACGIACGIACIAIGAIGIIIGIGCGAGIIGIGGGGACGACGACGIGG CTCCTATATCCCAAAACCCCCTTACCATCCCTACTACATACTCTCCCCCC
		CTAAGCCCCGACCACGCCCCCAGACCCCCGAGTCCTCCGGCTACCTCCGCCTCCAGCACGCCCCATCCCACC
		GGCATTGCAACCGTGCACGCCTTTCGACTATCCGCCTCTTGGCTGTGGAACATGTACAACAACGT
		GACAGCCGAGCTGGACGCCGGCGACTTAGAGCGACTTCCAGAACTGCACGCTCTGTCTTGCTGTC
		TGAAGGCCGTTTCGACTGCTGATGCCTCTGAATGCGTCGAGCGATGCCGACTTGCTTG
		CACGGATACATGTTGTCCTCTAACTTCCCTCTGATGTACGGCATGGTGACCGCCGCTTGTACTTA
		GATTGCCCAGTGTGTTGCTAATATCGAAAAACGACAGCGAACCGGAATGTCTTACGAGGATGCTT
		GGAACATGACCTCAGTGCAGCTTGTGTGTGTGTGCTAGCGAGGCCCACTGTCGAGCTTTCATCCTCCGG
		ACCTACTTCGAGGAGACCGAAAACCAAATCGGTTCCGTCTCTCCCGCTCTGCGAGCAGTGCTGCT
		GCAACTGGTCGATTTGTACGTTGTGTTTTGGGCTCTGCAGCGTGTCGGAGACCTTCTGAGATTCA
		CCTCTATCTCTGAGCGAGACATCGAGCAGCTTCAGTCTTGGTACGAGGATCTTCTGATCAAGCTG
		CGAGTCAACGCTGTCGGACTGGTCGACGCTTTCGACATCCGAGACGAGATCTTGAACTCTGCTCT
		CUGAGUL I AUGAUGUGUGAGU I I AUGAGUGUU I I A I GUA I GAAGUUU I I AAG I U I UUUU I GAAUG CTCA ACCTCTCA A ACC A CTCTTTCC ATA ACTATCTCA A CCCTTTC ATCCA CCCCT A A CCTCT A A
Sequence No. 6	Peroxisomal	ATGGAGTCTAAGGACTTTGACCCTGTGTCCGATGCTGAGTTGAAAGACTACTTCCCCGACCTCCC
	oxidase 49554	TTCTGGTCCTCTGGATAAGTTCCGAAAGAAGGCCACCTTCGACTGGAGACGAATGAAGCTAGTGT
	from Lobesia	ACGACTCTAAGCAGTCTATTGAGACCAAGGATAAGGTGTGGAAGTTCATGCTTGCT
	botrana	TTCAAGCACTCTGTCGCCACCCCACCCTGGATGAGCAGCGACAGATTGCTACCAAACGTATGTA
		TCTGCTGCATAACGCTGACCTGGTCCCTCTGGAGGAAATCGCTATGCATCCCCGACTCTTTCAGTC
		GIRACIGAGGCCATTITICATGTTCGACTCTTCGGCGGCGCGTTAAGCTGTCTGTACCTTCCGAA
		1 - 11-1111 ALE AAL AL AATTLELEET-ATTLELEE AL-AL AL-ATTALE ATT ALTALE ALTEATT-ATTL-ATTL-AL-LATTLEAT
		AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGATCGCCCACGGATCTAATGCTAAGGGCAT
		AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGACAGCATCGCCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCCAACCTACGACGTGGAGGGCCGATGTTTCGTCATGCACACCCCCCGACTTTGAAG
		AACGGCAAGATCGGCGGATCGCGGATCGGCAGACAGGATCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCGAACCTACGACGTGGAGGGCCGATGTTTCGTCATGCACACCCCCGGACTTTGAAG CTGCCAAGTGTTGGGTTGG
		AACGGCAAGATCGGCGGATCGCGGATCGGCAGACAGGATCACTACCACTTGATTGA
		ACGGCAAGATCGGCGGATCGGCAGACAGGACAGGATCACCACTTGATTGA
		ACGGCAAGACACACAACACACTCCGGCGATCGGCAGACAGGACAGGATCACCTCGCCACGGATCTAATGGCGAGCTGGCGA AACGGCAAGATCGGCGGATGCTTCGCCCTGGCCGAGGTCGCCCACGGATCTAATGGTAAGGGCAT GCGAACCACCGCCAACCTACGACGTGGGGGGGGGG
		ACGGCAACCACCACACACACATTCGCGGATCGGCAGACAGCATCACTACCACTTACTGAGGACTGCGAC AACGGCAAGATCGGCGGGATGCTTCGCCCTGACTGAGAGATCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGGGGGGGGG
		ACGGCAAGACACACAACAACATTCGCGGATCGGCCAGACAGGATCACCACGCACG
		ACGGCAAGATCGGCGGATCGTCGGCCGAGCAGGAGAGGATCACLACLACLACLTGATTGAGGAGTGGCAC AACGGCAAGATCGGCGGGATGCTTCGCCCTGACTGAGGATCGCCACGGATCTAATGGTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGGGGGGGGG
		AGTTCACCAACAACAACAATTCGCCGATCGGGCAGACAGCATCACTACCACTTACTGACGACTGCGAC AACGGCAAGATCGGCGGGATGCTTCGCCCTGACTGAGGATCGCCACGGATCTAATGGTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGGGGGGGGG
		ACGCAACAACAACAATTCGCCGATCGGCCAGACAGCATCACTACCACTTGATTGA
		ACGGCAAGAACAACAACAATTCGCCGATCGGCCAGACAGCATCACTACCACTTGATTGA
		ACGCAAGAACAACAACAATTCGCCGATCGGCCAGACAGCATCACTACCACTTGATGAGGACTGCGAC AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGAGTCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTTCGTCATGCCACGCCATTGTCTAAGGCAAT CTGCCAAGTGTTGGGTTGG
		ACGCAAGAACAACAACAATTCGCGGATCGGCCCGAACAGCATCACTACCACTTGACGACTGCGAC AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGAGTCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTTCGTCATGCCACGCCATTGTCTAAGGCACTGAAG CTGCCAAGTGTTGGGTTGG
		ACGCAAGAACAACAACAATTCGCGGATCGGCCCGAAGACAGCATCACTACCACTTGATGAGGACTGCGAC AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGAGTCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTCGTCGCCACGCCACCCGGACTTTGAAG CTGCCAAGTGTTGGGTTGG
		ACGCAAGAACACAATTCGCCGATCGGCCCTGACTGAGACAGCATCACTACCACTTGATGGAGGACTGCGAC AACGGCAAGATCGGCGGATGCTTCGCCCTGGCCTGACTGGAGACTCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTCGTCGCCACGCCATTGGACGCCATTGGAG CTGCCAAGTGTTGGGTTGG
		ACGGCAAGATCGGGCGGATCGGGCCGAGCAGACAGCATCACTACCACTTGATGAGGACTGCGAC AACGGCAAGATCGGGCGGATGTTCGCCCTGGCTGACTGAGATCGCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTCGTCGCCACGCCATCGGACGCCATCGGACGCCATCGGACGCATGGTTGGCTCCCTGGGTAGCCCATCGGCGCCATCGGCCATCGGCCATCGGCCATCGGCGACGACGACGACG ATCTCTAAAGGGCAAAAACCATGGTCTTCATTCCTTCGTGGTGCCCATCCGACGACGCACGGACGG
		ACGCAACAACAACAATTCGCCGATCGGCCCGAACAGCATCACTACCACTTGATTGA
		ACGGCAAGATCGGGCGGATCGGGCCGGACGGCAGACAGGATCACTACCACTTGATGGAGGACTGCGAC AACGGCAAGATCGGGCGGATGTTCGGCCCTGGCCGAGTTGGCGCCACGGATCTAATGGTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGGAGGGCCGATGTTCGTCGCCACGCCATGGCTAAGGCCAT CTGCCAAGTGTTGGGTTGG
		ACGCAAGAACAACAATATCGCGGATCGGGCAGACAGGATCACTACCACTAGATGGAGACTGCGAC AACGGCAAGATCGGGCGGATGTTCGGCCCTGGACTGAGAGTCGCCCACGGATCTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTCGGAGGGCCGATGTTCGTCGCCACGCCATTGCAAGGGCAT CTGCCAAGTGTTGGGTTGG
		ACGGCAAGATCGGGCGGATCTGGGCCGGACGGGACGCGCCCCGGACTTAATGGAGGACTGCGAC AACGGCAAGATCGGGCGGATGTTCGGCCCTGGACTGAGGTGCGCCCACGGATCTAATGGTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCGATGTTCGTCGCGCCACGCCGATGTTCACGCCGAGCTG ATCTCTAAGGGCAAAAACCATGGTCTTCATTCCTTCGTGGTGCCCATCCGAGACTCCTAAGACACT CCGACCTTTTGCAGGCGTGACTGTCGGAGACATCGGCGAGAGAGA
		ACGCAACAACAACAACAACCGCCGAGCCGAGCGAGACAGCATCACTACCTAC
		AACGGCAACAACAACAA ITCGCCGATCGCTCGACTGACATCACTACCTACCACTTAATGCTAAGGGCAT GCGAACCACCGCAACCTACGACGTGGAGGGCCCATGTTCGTCATGCACACCCCCGACTTTGAAG GCGAACCACCGCAACCTACGACGTGGGAGGGCCGATGTTCGTCATGCACACCCCCGACTTTGAAG GCGAACCACCGCAACCTTGGGTCGCTGGGTAAGTGTGCTACCCACGCCATTGTCTACGCCATGCTG ATCTCTAAGGGCAAAAACCATGGTCTTCATTCCTTCGTGGTGCCCACGGCCTTCGACAGGAGTGGACA ACGGTTTCCTGAAGGCGTGACTGTCGGAGACATCGGCGAGAGAGA
Sequence No. 7	Peroxisomal	AACGGCAACAACAACAACTCCGCCTGACTCGAGACAGCATCACTTCATTCA
Sequence No. 7	Peroxisomal oxidase from Anngrailus	AACGGCAAGATCGGCGGATGCTTCGCCCTGACTGAGACAGCATCACTTGCTTG

		GCAGCAGCTGAAGAAGGCCCACAACTGGTCTGACGAGGACGTCCACGTGGCCAACGACCTGGTGT CTGAGCCCACTCCTTACGGCCTGCACGCCTCTATGTTTCTGGTGACCCTGCGAGAGCAGGGCACCC CTGAGCAGCACAAGCTGTTCTACGAACGAGCCCGAAACTACGAGATCATCGGCTGCTACGCCCAG ACCGAGCTTGGCCACGGCTCTAACGTGCGAGGACTCGAGACTACCGCCACTTGGGACCCCCTTGA CCAGACCTTCATCATTCACTCTCCCACTCTGACCGCCTCTAAGTGGTGGATCGGCTCTCTGGGACG AACCGCCAACCACGCCGTGGTGATGGCCCACTGTACATCGGCGGCAAGAACTACGGACCCCATC CTTTCGTGGTGCAGATCCGAGACATGGAAACCACCAGCCTCTCGAGAACGTGACGTGGGCGAC ATCGGCCCCAAGTTCGGCTACAACACCATGGACAACGCCTTCTCGGGGACAACGGCTTCTCACAAGACTGGAGAA TCCCCACGTGAACATGCTGGCCCGATTCGCCCAGGTGGACAAGGCCACCAACAACATGGTGCAGAGAT CCCCACGTGAACATGCTGGCCCGATTCGCCCAGGTGGACAAGGCCACCAACAACATGGCGCGC GCGGCGTGCTCGCCCGAGGCGTGACCATTGCCGTGCGATACTGCGCCGCGCGCG
		CGCCGTCCGACTGGTGGACTCTTGGAAGATCCCCGACTGGCAGCTGGACTCTGCCCTGGGCCGATC TGACGGCGACGTGTACCCCGACCTGTTCAAGCGAGCCTCTATGCAGAACCCCGGTGAACGACCTCG TGTTCGACCCCTATCCTTGGAACGACGACGTCCTGAAGAACGCCGGTGAGATCAAGTCTAAGCTG
		TAG
Sequence No. 8	Peroxisomal oxidase from <i>Cucurbita</i> <i>maxima</i>	ATGGCCGCTGGCAAGGCCAAGGCTAAGATCGAGTGGACATGGGACTCTCTGTCTCTGTACATGCG AGGCAAGCACCGAGAGATCCAAGAGCGAGTGTTCGAGCACTGGGCAGCAGCCGAGCCGAGCGCGGCGGCGGCGGCGGCGGC
Sequence No. 9	Peroxisomal oxidase from Homo sapiens	ATGAACCCCGACCTGCGACGAGAGCGAGACCTCTGCCTCTTCCAACCCCGAGCTGCTGACCCACATC CTGGACGCCTCTCCCGAAAAGACCCGACGACGACGAGAAATCGAGAACATGATCCTGAACGACCC CGACTTCCAGCACGAGGACCTGAACTTTCTGACCCGATCTCAGGAACATGGAGCGGGCGG

		CGTGCGACACCAGTCTGAGATCAAGCCCGGCGAGCCTGAGCCTCAGATCCTGGACTTTCAGACCC
		AGCAGTACAAGCTGTTCCCTCTGCTGGCCACCGCTTACGCCTTCCAGTTCGTGGGCGCCTACATGA
		AGGAAACCTACCATCGAATCAACGAAGGCATCGGCCAGGGCGACCTGTCTGAGCTGCCCGAACTG
		CACGCCCTGACCGCCGGACTGAAGGCTTTCACCTCTTGGACCGCCAACACCGGCATCGAGGCCTGC
		CGAATGGCCTGTGGCGGCCACGGCTACTCTCACTGCTCTGGACTGCCCAACATCTACGTGAACTTC
		ACCCCTTCGTGTACCTTCGAGGGCGAGAACACCGTGATGATGCTGCAGACCGCTCGATTCCTCAT
		ATCTCTCAGAACGCCGGCCACTTCCTGCAGGGCTCTATCATGACTGAGCCCCAGATTACCCAGGT
		TCGACTTTCAGGACGTGACCCTGGGCTCTGTGCTGGGCCGATACGACGGCAACGTGTACGAGAAC
		CTGTTCGAGTGGGCCAAGAACTCGCCCCTGAACAAGGCCGAGGTGCACGAGTCTTACAAGCACCT
		GAAGTCTCTGCAGTCTAAGCTGGACCAGATTACTTCTGTGGGATCTTCTTCGAAGCTGTAG
Sequence No.	Peroxisomal	ATGACCGAGGTGGTGGACCGAGCCTCTTCTCCCGCCTCTCCTGGCTCTACCACCGCCGCTGCCGAC
10	oxidase from	GGCGCCAAGGTGGCCGTCGAGCCTCGAGTGGACGTGGCCGCTCTGGGCGAGCAGCTCCTCGGCCG
	Paenarthrobact	ATGGGCCGACATCCGACTGCACGCCCGAGATCTGGCCGGACGAGAGGTGGTGCAGAAGGTCGAGG
	er ureafaciens	GACTGACCCACACCGAGCACCGATCTCGAGTGTTCGGCCAGCTGAAGTACCTGGTGGACAACAAC
		GCCGTGCACCGAGCTTTCCCTTCTCGACTCGGCGGATCTGACGACCACGGCGGCAACATTGCCGGC
		TTCGAGGAACTGGTGACTGCTGACCCCTCGCTGCAGATCAAGGCCGGCGTCCAGTGGGGGCCTGTT
		CGGCTCTGCCGTGATGCACCTGGGCACCCGAGAGCACCACGACAAGTGGCTGCCCGGCATCATGT
		CTCTCGAGATCCCCGGCTGCTTCGCCATGACCGAGACTGGCCACGGCTCTGACGTGGCCTCTATCG
		CCACCACCGCCACCTACGACGAAGAGACTCAAGAGTTCGTGATCGACACACCCTTCCGAGCCGCCT
		GGAAGGACTACATCGGCAACGCCGCCAACGACGGCCTGGCCGCCGTCGTGTTCGCTCAGCTGATC
		ACCCGAAAGGTGAACCACGGCGTCCACGCCTTCTACGTGGACCTGCGAGATCCCGCCACCGGCGA
		CTTTCTGCCCGGAATCGGCGGCGAGGACGACGGCATCAAGGGCGGCCTGAACGGCATCGACAACG
		GGACACCCTGCAAGAGTGCCGAGAGGCCTGCGGCGGGGGGGG
		CCTCTCTGCGAGCTGACCTGGACGTGTACGTGACCTTCGAGGGCGACACACGTGCTGCTGCAG
		CTGGTGGCCAAGCGACTGCTGGCCGACTACGCCAAGGAATTCCGAGGCGCCCAACTTCGGCGTGCT
		GGCCCGATACGTCGTGGACCAGGCCGCTGGCGTGGCTCTGCACCGAACCGGCCTGCGACAGGTGG
		CCCAGTTCGTGGCCGACTCCGGCTCTGTGCAGAAGTCTGCCCTGGCTCTGCGAGATGAGGAAGGC
		CAGCGAACCCTGCTGACCGACCGAGTGCAGTCTATGGTGGCCGAGGTGGGCGCTGCCCTGAAGGG
		CGCTGGCAAGCTGCCCCAGCACCAGGCTGCTGCCCTGTTCAACCAGCATCAGAACGAGCTGATCG
		AGGCCGCTCAGGCCCACGCCGAGCTGCTCCAGTGGGAAGCCTTCACCGAGGCTCTGGCCAAGGTC
		GACGACGCCGGCACCAAGGAAGTGCTGACCCGACTGCGGGACCTGTTCGGACTGTCTCGATTGA
		GAAGCACCTGTCTTGGTATCTGATGAACGGCCGACTGTCTATGCAGCGGGGACGAACCGTGGGCA
		CCTACATCAACCGACTGCTCGTGAAGATTCGACCCCACGCTCTGGACCTGGTCGACGCCTTCGGCT
		ACGGCGCTGAGCATCTGCGAGCCGCCATTGCCACCGGTGCCGAGGCCACTCGACAGGACGAGGCC
		CGAACCTACTTCCGACAGCAGCGAGCCTCTGGATCTGCCCCTGCCGACGAAAAGACCCTGCTGGCC
		ATTAAGGCCGGCAAGTCCCGAGATCAGATTACCTCTGTGGGATCTTCTTCGAAGCTGTAG
Sequence No.	Peroxisomal	
11	oxidase from	
	Rattus	
	norvegicus	
		ACCGCCACTTACGACCCCAAGACTCAAGAGTTCATCCTGAACTCTCCCACCGTGACCTCTATCAAG
		TGGTGGCCCGGTGGCCTGGGCAAGACCTCTAACCACGCCATCGTGCCGGCCCAGCTGATTACCCA
		GGGCGAGTGCTACGGCCTGCACGCCTTCGTGGTGCCCATCCGAGAGATCGGAACCCACAAGCCAC
		TGCCTGGCATCACCGTGGGCGACATCGGCCCCAAGTTCGGCTACGAGGAAATGGACAACGGCTAC
		CTGAAGATGGACAACTACCGAATTCCTCGAGAGAACATGCTGATGAAGTACGCCCAGGTGAAGCC
		CGACGGAACCTACGTGAAGCCCCTGTCTAACAAGCTGACCTACGGAACCATGGTGTTCGTGCGAT
		CTTTCCTGGTGGGCAACGCCGCTCAGTCTCTGTCTAAGGCCTGCACCATTGCCATCCGATACTCTG
		CCGTGCGACGACAGTCTGAGATCAAGCAGTCTGAGCCCGAGCCTCAGATCCTGGACTTTCAGACC
		CAGCAGTACAAGCTGTTCCCTCTGCTGGCCACCGCCTACGCCTTCCACTTCGTGGGCCGATATATG
		AAGGAAACCTACCTGCGAATCAACGAGTCTATCGGCCAGGGCGACCTGTCTGAGCTGCCCGAGCT
		GCACGCCCTGACCGCCGGACTGAAGGCTTTCACCACCTGGACCGCCAACGCCGGCATCGAGGAAT
		GCCGAATGGCCTGTGGCGGCCACGGCTACTCTCACTCCTCTGGCATCCCCAACATCTACGTGACCT
		TLAUTULUGUUTGUAUUTTUGAGGGTGAGAACACCGTGATGATGCTGCAGACCGCTCGATTCCTG
		A I GAAGATUTALGALLAGGTGUGATUTGGUAAGUTGGTUGGUGGUATGGTGTUTTAUUTGAAUGA
1	1	inite i dentine de la

		CCTGGTGCGAGCTTCTGAGGCCCACTGCCACTACGTGGTGGTGAAGGTGTTCTCTGACAAGCTGC
		CCAAGATCCAGGACAAGGCTGTCCAGGCCGTGCTGCGAAACCTGTGCCTGCTGTACTCTGTAC
		GGAATCTCTCAGAAGGGCGGCGACTTCCTCGAGGGCTCTATCATCACCGGCGCTCAGCTGTCTCA
		GGTCAACGCTCGAATCCTCGAGCTGCTGACCCTGATTCGACCCAACGCCGTGGCTCTGGTGGACG
		CTTTCGACTTCAAGGACATGACCCTGGGCTCTGTGCTGGGACGATACGACGGCAACGTGTACGAG
		AACCTCTTCGAGTGGGCCAAGAAGTCTCCCCTGAACAAGACCGAGGTGCACGAGTCTTACCACAA
		GCACCTGAAGCCTCTGCAGTCTAAGCTGGACCAGATTACCTCCGTGGGATCTTCTTCGAAGCTGT
		AG
Sequence No.	Humanized	ATGGTGAGCAAGCAGATCCTGAAGAACACCTGCCTGCAGGAGGTGATGAGCTACAAGGTGAACCT
12	renilla green	GGAGGGCATCGTGAACAACCACGTGTTCACCATGGAGGGCTGCGGCAAGGGCAACATCCTGTTCG
	fluorescent	GCAACCAGCTGGTGCAGATCCGCGTGACCAAGGGCGCCCCCTGCCCTTCGCCTTCGACATCGTG
	protein	AGCCCCGCCTTCCAGTACGGCAACCGCACCTTCACCAAGTACCCCAACGACATCAGCGACTACTT
		CATCCAGAGCTTCCCCGCCGGCTTCATGTACGAGCGCACCCTGCGCTACGAGGACGGCGGCCTGG
		TGGAGATCCGCAGCGACATCAACCTGATCGAGGACAAGTTCGTGTACCGCGTGGAGTACAAGGGC
		AGCAACTTCCCCGACGACGGCCCCGTGATGCAGAAGACCATCCTGGGCATCGAGCCCAGCTTCGA
		GGCCATGTACATGAACAACGGCGTGCTGGTGGGGGGGGGG
		GCAAGTACTACAGCTGCCACATGAAGACCCTGATGAAGAGCAAGGGCGTGGTGAAGGAGTTCCCC
		TCCTACCACTTCATCCAGCACCGCCTGGAGAAGACCTACGTGGAGGACGGCGGCTTCGTGGAGCA
		GCACGAGACCGCCATCGCCCAGATGACCAGCATCGGCAAGCCCCTGGGCAGCCTGCACGAGTGGG
		TGTAA
Sequence No.	mCherry	ATGGTGTCTAAGGGCGAAGAGGACAACATGGCCATCATCAAGGAATTCATGCGATTCAAGGTGC
13		ACATGGAAGGCTCTGTGAACGGCCACGAGTTCGAGATCGAAGGCGAAGGCGAGGGACGACCCTAC
		GAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGACCCCTGCCTTTCGCCTGGGACAT
		TCTGTCTCCCCAGTTCATGTACGGCTCTAAGGCCTACGTGAAGCACCCCGCCGACATTCCCGACTA
		CCTGAAGCTGTCGTTCCCCGAGGGCTTCAAGTGGGAGCGAGTGATGAACTTCGAGGACGGCGGCG
		TGGTGACCGTGACTCAGGACTCTTCGCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGA
		GGCACCAACTTTCCCTCTGACGGCCCCGTGATGCAAAAGAAGACCATGGGCTGGGAAGCCTCTTC
		TGAGCGAATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGCAGCGACTGAAGCTCAAGG
		ACGGTGGCCACTACGACGCCGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTCCAGCTGCCT
		GGCGCCTACAACGTGAACATCAAGCTGGACATCACCTCTCACAACGAGGACTACACCATCGTCGA
		GCAGTACGAGCGAGCCGAGGGCCGACACTCTACCGGCGGCATGGACGAGCTGTACAAGTAG

Supplementary table S3. Biobricks used in this study

ID	Description	Forward	Reversed	Template DNA	Reference
		primer	primer	_	
BB8302	{-PrTEF1intron	PR-18928	PR-18975	Genomic DNA of	This study
				ST4840	
BB2068	Har_FAR_Ylop->	PR-18066	PR-16595	Sequence No. 1	This study
BB2720	{-PrTEF1intron_USER_forfusion	PR-18930	PR-18214	Genomic DNA of	This study
				ST4840	
BB8644	PrGPD_forfusion-}	PR-23004	PR-22213	Genomic DNA of	This study
				ST4840	
BB8640	Dmd9{-PrTEF1intron_forfusion	PR-19018	PR-18930	Sequence No. 2	This study
BB2693	{-Lbo_PPTQ	PR-21723	PR-21724	Sequence No. 3	This study
BB1135	Vector backbone	See ref.	See ref.	See ref.	Holkenbrink
					2018
BB8769	{-Ase_POX	PR-23435	PR-23436	Sequence No. 4	This study
BB2709	Lbo31670->	PR-21755	PR-21756	Sequence No. 5	This study
BB1558	PrExp-}	PR-15521	PR-15522	Genomic DNA of	This study
				ST4840	
BB2710	Lbo49554->	PR-21757	PR-21758	Sequence No. 6	This study
BB1635	PrtRNA-Gly	See ref.	See ref.	See ref.	Holkenbrink
					2018
BB1636	crRNA-TRPR	See ref.	See ref.	See ref.	Holkenbrink
					2018
BB8516	{-Yli_POX2	PR-22827	PR-22828	Genomic DNA of	This study
				ST4840	
BB8517	{-Yli_POX3	PR-22829	PR-22830	Genomic DNA of	This study
				ST4840	
BB8519	{-Yli_POX5	PR-22833	PR-22834	Genomic DNA of	This study
	(PP 00011	PP 00010	ST4840	
BB8523	{-Ani_POX	PR-22841	PR-22842	Sequence No. 7	This study
BB8524	{-Cma_POX	PR-22843	PR-22844	Sequence No. 8	This study
BB8525	{-Hsa_POX	PR-22845	PR-22846	Sequence No. 9	This study
BB8526	{-Pur_POX	PR-22847	PR-22848	Sequence No. 10	This study
BB8527	{-Rno_POX	PR-22849	PR-22850	Sequence No. 11	This study
BB1688	->PrTEF1intron	See ref.	See ref.	See ref.	Petkevicius
					2021

BB1740	Har_FAR_codoptYL	See ref.	See ref.	See ref.	Holkenbrink 2020
BB9309	PrTEF1intron_HarFAR_Per2	PR-10595	PR-24919	pBP8236	This study
BB8682	IntD_2_dwn	PR-23172	PR-23173	Genomic DNA of ST4840	This study
BB8681	IntD_2_up	PR-23171	PR-23170	Genomic DNA of ST4840	This study
BB2313	Fas2 (I1220F)	See ref.	See ref.	See ref.	Petkevicius 2021
BB8679	IntE_7_up	PR-23167	PR-23166	Genomic DNA of ST4840	This study
BB8680	IntE_7_dwn	PR-23168	PR-23169	Genomic DNA of ST4840	This study
BB1631	TPex20-TLip2	See ref.	See ref.	See ref.	Holkenbrink 2018
BB8386	IntF_5_Up	PR-22532	PR-22533	Genomic DNA of ST4840	This study
BB8387	IntF_5_Down	PR-22534	PR-22535	Genomic DNA of ST4840	This study
BB10144	PrTEF1intronHarFARforhrGFP	PR-10595	PR-26919	pBP8236	This study
BB10145	hrGFPforHarFAR	PR-26920	PR-15506	Sequence No. 12	This study
BB10146	hrGFPforHarFAR_Per2	PR-26920	PR-26921	Sequence No. 12	This study
BB2093	PrTEF1intron_USER-}	See ref.	See ref.	See ref.	Petkevicius 2021
BB10154	YliPOX3formCherry	PR-24936	PR-26932	Genomic DNA of ST4840	This study
BB10155	YliALE1formCherry	PR-26926	PR-26933	Genomic DNA of ST4840	This study
BB10156	mCherryforYliPOX3	PR-26934	PR-26936	Sequence No. 13	This study
BB10157	mCherryforYliALE1	PR-26935	PR-26936	Sequence No. 13	This study

Supplementary table S4. Plasmids used in this study

ID	Description	Parent plasmid	Biobricks/primers	Reference
pCfB6630	pNat-YLgRNA3_IntC_3	See ref.	See ref.	Holkenbrink 2018
pBP8754	pIntF_3-Ase_POX_GeneArt{-PrEXP	pBP8009	BB2721, BB8769	This study
pBP8627	pIntD_2-Dmd9{-PrTEF1intron-	pBP8620	BB8640, BB2720,	This study
_	PrGPD-}HarFAR		BB8644, BB2068	
pBP8400	pIntC_3-TPex20-PrEXP-}Lbo31670-	pCfB6371	BB2709, BB1558	This study
	TLip2			
pBP8401	pIntC_3-TPex20-PrEXP-}Lbo49554-	pCfB6371	BB2710, BB1558	This study
	TLip2			
pBP8802	pIntD_2-Lbo_PPTQ{-PrTEF1intron-	pBP8620	BB2693, BB2720,	This study
	PrGPD-}HarFAR		BB8644, BB2068	
pCfB7088	pNat-YLgRNA1_Fas2 (AA1220)	See ref.	See ref.	Holkenbrink 2020
pBP8900	pHph_YLgRNA5_IntE_4	pCfB3431	BB1635, BB1636, PR-	This study
			23285, PR-23286	
pBP8340	pIntC_3-TPex20-Yli_POX2{-PrTEF1-	pCfB6371	BB8516, BB8302	This study
	TLip2			
pBP8341	pIntC_3-TPex20-Yli_POX3{-PrTEF1-	pCfB6371	BB8517, BB8302	This study
	TLip2			
pBP8343	pIntC_3-TPex20-Yli_POX5{-PrTEF1-	pCfB6371	BB8519, BB8302	This study
	TLip2			
pBP8347	pIntC_3-TPex20-Ani_POX{-PrTEF1-	pCfB6371	BB8523, BB8302	This study
	TLip2			
pBP8348	pIntC_3-TPex20-Cma_POX{-PrTEF1-	pCfB6371	BB8524, BB8302	This study
	TLip2			
pBP8349	pIntC_3-TPex20-Hsa_POX{-PrTEF1-	pCfB6371	BB8525, BB8302	This study
	TLip2			
pBP8350	pIntC_3-TPex20-Pur_POX{-PrTEF1-	pCfB6371	BB8526, BB8302	This study
	TLip2			
pBP8351	pIntC_3-TPex20-Rno_POX{-PrTEF1-	pCfB6371	BB8527, BB8302	This study
	TLip2			
pBP8003	pNat-YLgRNA4_IntF_3	pCfB3405	BB1635, BB1636, PR-	This study
		0750.407	22039, PR-22040	
pBP8623	pNat_YLgRNA1_IntD_2	pCfB3405	BB8736	This study
pBP8576	pHph_YLgRNA1_IntD_2	pCfB3431	BB1635, BB1636, PR-	This study
			23192, PR-23193	

pBP8032	pHph-YLgRNA3_IntC_3	pCfB3431	BB1635, BB1636, PR- 18239, PR-18240	This study
pBP8236	pIntE_4-PrTEF1intron->HarFAR	pCf6679	BB1688, BB1740	This study
pBP9438	pIntE_4_PrTEF1intron_HarFAR_Per2	pCf6679	BB9309	This study
pBP8009	pIntF_3-TPex20-TLip2		BB1135, BB1631,	This study
			BB8031, BB1480	
pBP6371	pIntC_3-TPex20-TLip2	See ref.	See ref.	Holkenbrink 2018
pBP3405	pORI1001-Nat-CEN1-USER	See ref.	See ref.	Holkenbrink 2018
pBP3431	pORI1001-Hphsyn-CEN1-USER	See ref.	See ref.	Holkenbrink 2020
pBP6679	pIntE_4-TPex20-TLip2	See ref.	See ref.	Holkenbrink 2018
pBP8620	pIntD_2-TPex20-TLip2		BB1135, BB8682,	This study
			BB8681	
pBP8575	pNat_YLgRNA1_IntE_7		BB1635, BB1636, PR-	This study
			23190, PR-23191	
pBP8645	pHph_YLgRNA4_IntF_5		BB1635, BB1636, PR-	This study
			23127, PR-23128	
pBP8662	pIntE_7-TPex20-TLip2		BB1135 ,BB8679,	This study
			BB8680, BB1631	
pBP8263	IntF_5_Up_TPex20-USER-		BB1135 ,BB8386,	This study
	TLip2_IntF_5_Down		BB1631, BB8387	
pBP10672	pIntE_7-TPex20-	pBP8662	BB10144, BB10145	This study
	PrTEF1intron_HarFAR_hrGFP-TLip2			
pBP10669	pIntE_7-TPex20- PrTEF1intron	pBP8662	BB10144, BB10146	This study
	_HarFAR_hrGFP_Per2-TLip2			
pBP10676	IntF_5_Up_TPex20- PrTEF1intron	pBP8263	BB2093, BB10154,	This study
	_YliPOX3_mCherry-		BB10156	
	TLip2_IntF_5_Down			
pBP10677	IntF_5_Up_TPex20- PrTEF1intron	pBP8263	BB2093, BB10155,	This study
	_YliALE1_mCherry-		BB10157	
	TLip2_IntF_5_Down			

Supplementary table S5. Strains used in this study

ID	Relevant features	Parent strain	Added elements	Reference/source
ST4840	Wild-type Yarrowia lipolytica			Agricultural Research
				Service (NRRL, USA)
ST6629	See ref.	See ref.	See ref.	Holkenbrink 2020
ST8524	See ref.	See ref.	See ref.	Petkevicius 2021
ST9138	ΔPOX1-6	See ref.	See ref.	Patent application
				W0/2020/169389
ST9199	ΔPOX1-6, IntC_3-Yli_POX2{-TEF1	ST9138	pCfB6630, pBP8340	This study
ST9200	ΔPOX1-6, IntC_3-Yli_POX3{- TEF1	ST9138	pCfB6630, pBP8341	This study
ST9202	ΔPOX1-6, IntC_3-Yli_POX5{- TEF1	ST9138	pCfB6630, pBP8343	This study
ST9206	ΔPOX1-6, IntC_3-Ani_POX{- TEF1	ST9138	pCfB6630, pBP8347	This study
ST9207	ΔPOX1-6, IntC_3-Cma_POX{- TEF1	ST9138	pCfB6630, pBP8348	This study
ST9208	ΔPOX1-6, IntC_3-Hsa_POX{- TEF1	ST9138	pCfB6630, pBP8349	This study
ST9209	ΔPOX1-6, IntC_3-Pur_POX{- TEF1	ST9138	pCfB6630, pBP8350	This study
ST9210	ΔPOX1-6, IntC_3-Rno_POX{- TEF1	ST9138	pCfB6630, pBP8351	This study
ST9284	ΔPOX1-6, IntF_3-Ase_POX{-PrEXP	ST9138	pBP8754, pBP8003	This study
ST9294	ΔPOX1-6, IntD_2-Dmd9{-	ST9138	pBP8627, pBP8623	This study
	PrTEF1intron-PrGPD-}HarFAR			
ST9295	ΔPOX1-6, IntC_3-Yli_POX2{-PrEXP,	ST9199	pBP8627, pBP8576	This study
	IntD_2-Dmd9{-PrTEF1intron-			
	PrGPD-}HarFAR			
ST9296	ΔPOX1-6, IntC_3-Yli_POX3{-PrEXP,	ST9200	pBP8627, pBP8576	This study
	IntD_2-Dmd9{-PrTEF1intron -			
	PrGPD-}HarFAR			
ST9297	ΔPOX1-6, , IntC_3-Yli_POX5{-PrEXP	ST9202	pBP8627, pBP8576	This study
	IntD_2-Dmd9{- PrTEF1intron-			
	PrGPD-}HarFAR			
ST9298	ΔPOX1-6, IntC_3-Ani_POX{-PrEXP,	ST9206	pBP8627, pBP8576	This study
	IntD_2-Dmd9{-PrTEF1intron-			
	PrGPD-}HarFAR			
ST9299	ΔPOX1-6, IntC_3-Cma_POX{-PrEXP,	ST9207	pBP8627, pBP8576	This study
	IntD_2-Dmd9{- PrTEF1intron-			
	PrGPD-}HarFAR			
ST9300	ΔPOX1-6, IntC_3-Hsa_POX{-PrEXP,	ST9208	pBP8627, pBP8576	This study
	IntD_2-Dmd9{- PrTEF1intron-			
	PrGPD-}HarFAR			

ST9301	ΔPOX1-6, IntC_3-Pur_POX{-PrEXP,	ST9209	pBP8627, pBP8576	This study
	IntD_2-Dmd9{- PrTEF1intron-			
	PrGPD-}HarFAR	277.0.2.1.0		
ST9302	ΔPOX1-6, IntC_3-Rno_POX{-PrEXP,	ST9210	pBP8627, pBP8576	This study
	IntD_2-Dmd9{- PrTEF1intron-			
670220	PrGPD-}HarFAR	CT0204		This standay
519329	ΔPUX1-6, IIILF_3-ASE_PUX{-PIEXP,	519284	рвр8627, рвр8576	This study
	PrCPD_\HarFAR			
ST9347	APOX1-6 IntD 2-Dmd9{-	ST9294	nBP8400 nBP8032	This study
019017	PrTEF1intron-PrGPD-}HarFAR.	517271	pb10100, pb10002	This study
	IntC_3-PrEXP-}Lbo_31670			
ST9348	ΔPOX1-6, IntD_2-Dmd9{-	ST9294	pBP8401, pBP8032	This study
	PrTEF1intron-PrGPD-}HarFAR,			
	IntC_3-PrEXP-}Lbo_49554			
ST9314	ΔΡΟΧ1-6, ΔΡΟΧ1-6, IntD_2-	ST9138	pBP8802, pBP8623	This study
	Lbo_PPTQ{- PrTEF1intron -PrGPD-			
	}HarFAR	2000 1 0 0		
\$19315	ΔPOX1-6, IntC_3-YII_POX2{-PrEXP,	ST9199	pBP8802, pBP8576	This study
	IntD_2-Lbo_PPTQ{- PrTEF1intron-			
ST0216	ADOVI 6 IntC 2 VI; DOV2(DrEVD	570200	pPD9902 pPD9576	This study
319310	$\Delta POA1-0$, $\Pi IIIC_3-\Pi POA3\{-PIEAP$, IntD 2-I ha PPTOL PrTEF1 intron-	519200	рвг8802, рвг8376	This study
	PrGPD-}HarFAR			
ST9317	ΔPOX1-6. IntC 3-Yli POX5{-PrEXP.	ST9202	pBP8802, pBP8576	This study
	IntD 2-Lbo PPTQ{- PrTEF1intron-		P== 000-, P== 000 0	
	PrGPD-}HarFAR			
ST9318	ΔPOX1-6, IntC_3-Ani_POX{-PrEXP,	ST9206	pBP8802, pBP8576	This study
	IntD_2-Lbo_PPTQ{- PrTEF1intron-			
	PrGPD-}HarFAR			
ST9319	ΔPOX1-6, IntC_3-Cma_POX{-PrEXP,	ST9207	pBP8802, pBP8576	This study
	IntD_2-Lbo_PPTQ{-PrTEF1intron-			
CTT0220	PrGPD-}HarFAR	CTT0 200		
519320	ΔPOX1-6, Intt_3-HSa_POX{-PTEXP, IntD 2 Lbo DDTO(DrTEE1introp	519208	рвр8802, рвр8576	This study
	PrCPD-\HarFAR			
ST9321	APOX1-6. IntC. 3-Pur. POX{-PrEXP.	ST9209	nBP8802, nBP8576	This study
017021	IntD 2-Lbo PPTO{- PrTEF1intron-	517207	pb10002, pb10070	This study
	PrGPD-}HarFAR			
ST9322	ΔPOX1-6, IntC_3-Rno_POX{-PrEXP,	ST9210	pBP8802, pBP8576	This study
	IntD_2-Lbo_PPTQ{- PrTEF1intron-			
	PrGPD-}HarFAR			
ST9330	ΔPOX1-6, IntF_3-Ase_POX{-PrEXP,	ST9284	pBP8802, pBP8576	This study
	IntD_2-Lbo_PPTQ{- PrTEF1intron-			
CTO 2 TO	PrGPD-}HarFAR	CTTO 214		
519350	ΔPUXI-6, INTD_2-LD0_PPIQ{- DrTEF1intron_DrCDD_HarEAD	519314	рвр8400, рвр8032	This study
	IntC 3-PrFXP-31670-31670			
ST9351	APOX1-6. IntD 2-Lbo PPTO{-	ST9314	nBP8401, nBP8032	This study
017001	PrTEF1intron -PrGPD-}HarFAR.	017011	p210101, p21000 2	into occury
	IntC_3-PrEXP-}Lbo_49554			
ST10313	ΔPOX1-6, IntD_2-Dmd9{-	ST9347	pCfB7088, BB8908	This study
	PrTEF1intron -PrGPD-}HarFAR,		_	
	IntC_3-PrEXP-}Lbo_31670, FAS2			
	(I1220F)			
ST10383	ΔPOX1-6, IntD_2-Dmd9{-	STST10313	pBP8900, pBP8236	This study
	PrTEFIIntron -PrGPD-}HarFAR,			
	(11220F) IntE A PrTEF1intron -			
	>HarFAR			
ST10384	ΔPOX1-6. IntD 2-Dmd9{-	STST10313	pBP8900, pBP9438	This study
	PrTEF1intron -PrGPD-}HarFAR,		P== 0.000, P== 0.000	
	IntC_3-PrEXP-}Lbo_31670, FAS2			
	(I1220F), IntE_4_PrTEF1intron			
	_HarFAR_Per2			
ST10314	ΔPOX1-6, IntD_2-Lbo_PPTQ{-	ST9350	pCfB7088, BB8908	This study
	PrTEF1intron -PrGPD-}HarFAR,			
	IntC_3-PrEXP-}Lbo_31670, FAS2			
CT10207	(II220F)	CT10214		This study
311038/	DrUAI-0, IIIU_2-LD0_PPIQ{- PrTFF1intron_PrCPD_1HarEAP	5110314	рогозоо, рвго236	This study
	IntC 3-PrEXP-}Lbo 31670. FAS2			

	(I1220F), IntE_4- PrTEF1intron -			
	>HarFAR			
ST10388	ΔPOX1-6, IntD_2-Lbo_PPTQ{-	ST10314	pBP8900, pBP9438	This study
	PrTEF1intron -PrGPD-}HarFAR,			
	IntC_3-PrEXP-}Lbo_31670, FAS2			
	(I1220F), IntE_4- PrTEF1intron -			
	>HarFAR_Per2			
ST12413	ΔPOX1-6, IntE_7- PrTEF1intron	ST9138	pBP8575, pBP10672	This study
	_HarFAR_hrGFP			
ST12410	ΔPOX1-6, IntE_7- PrTEF1intron	ST9138	pBP8575, pBP10669	This study
	_HarFAR_hrGFP_Per2			
ST12433	ΔPOX1-6, IntE_7- PrTEF1intron	ST12413	pBP8645, pBP10676	This study
	_HarFAR_hrGFP, IntF_5-			
	PrTEF1intron_YliPOX3_mCherry-			
ST12434	ΔPOX1-6, IntE_7- PrTEF1intron	ST12413	pBP8645, pBP10677	This study
	_HarFAR_hrGFP, IntF_5-			-
	PrTEF1intron_YliALE1_mCherry-			
ST12424	ΔPOX1-6, IntE_7- PrTEF1intron	ST12410	pBP8645, pBP10676	This study
	_HarFAR_hrGFP_Per2, IntF_5-			
	PrTEF1intron_YliPOX3_mCherry-			
	TLip2_IntF_5_Down			
ST12425	ΔPOX1-6, IntE_7- PrTEF1intron	ST12410	pBP8645, pBP10677	This study
	_HarFAR_hrGFP_Per2, IntF_5-		_	
	PrTEF1intron_YliALE1_mCherry-			
	TLip2_IntF_5_Down			

Supplementary materials and methods relevant for generation of data presented in supplementary table S6 and supplementary figure S7.

Y. lipolytica strains were inoculated into 37.5 mL of YPG media in 250 mL shake flasks at OD₆₀₀=0.2 and cultivated for 22 h at 28°C, shaken at 300 rpm at 5 cm orbit cast. Cell growth over time was monitored by measuring optical density using GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Scientific). After 22 h of growth, cultivation broth was transferred into 50 mL falcon tube, centrifuged for 5 min at room temperature at 3000xg. The supernatant was discarded, and the cells resuspended in 20 mL production medium. Resuspended cells were transferred back to shake flasks and 0.4% (v/v) of 14:Me supplied. For cell dry weight and glycerol measurements, 1 mL cultivation broth was taken and centrifuged for 5 min at room temperature at 12500xg. Supernatant was used for glycerol measurements. Cell pellet was resuspended in 1 mL 70% ethanol and centrifuged for 5 min at room temperature at 12500xg. Liquid was removed and pellet was kept for 72 h in the 70°C degrees oven before weight measurements. Glycerol was measured using Randox GY105 glycerol assay. Quantification of 14:Me and Z7-12:OH was performed using GC-FID under the same settings as for data presented in Figures S1 and S2. Quantification of Z9-12:OH was performed under the same settings as for data presented in Figure 2.

Supplementary table S6. Titers and specific yields (g of product/g of dry weight) of Z7-12:OH, Z9-12:OH and Z7-14:OH at the end of shake flask cultivations (52h after media exchange).

Product Strain	27-12:ОН	Z9-12:ОН	Z7-14:OH
	1.13±0.05 mg/L	0 mg/L	0.15±0.04 mg/L
ST10384	Y _{xp} =4.8x10 ⁻⁵ ±4.4x10 ⁻⁶	Y _{xp} =0	Y _{xp} =6.4x10 ⁻⁶ ±2.1x10 ⁻⁶
	0 mg/L	0.33±0.07 mg/L	0.13±0.06 mg/L
ST10388	Y _{xp} =0	Y _{xp=} 1.1x10 ⁻⁵ ±1.9x10 ⁻⁶	Y _{xp=} 4.3x10 ⁻⁶ ±2.2x10 ⁻⁶

Supplementary references:

Holkenbrink C, Dam MI, Kildegaard KR *et al*. EasyCloneYALI: CRISPR/Cas9-Based Synthetic Toolbox for Engineering of the Yeast *Yarrowia lipolytica*. *Biotechnol J* 2018; 13:1–8.

Holkenbrink C, Ding BJ, Wang HL, *et al.* Production of moth sex pheromones for pest control by yeast fermentation. *Metab Eng* 2020, DOI:10.1101/2020.07.15.205047.

Petkevicius K, Koutsoumpeli E, Betsi PC *et al.* Biotechnological production of the European corn borer sex pheromone in the yeast *Yarrowia lipolytica*. *Biotechnol J* 2021, DOI:10.1002/biot.202100004



Chromatogram number	Strain ID	FAD	FAR	Peroxisomal oxidase
1	ST9294			-
2	ST9295			YliPOX2
3	ST9296			YliPOX3
4	ST9297			YliPOX5
5	ST9298		Dmd9 HarFAR	AniPOX
6	ST9299			CmaPOX
7	ST9300			HsaPOX
8	ST9301			PurPOX
9	ST9302			RnoPOX
10	ST9329			AsePOX
11	ST9347			Lbo_31670
12	ST9348			Lbo_49554

Figure S1. Fatty alcohol profiles obtained from the Y. lipolytica strains containing Dmd9, HarFAR and different POXes. Cultivation media was supplemented with 0.24% (v/v) of 14:Me.



Figure S2. Fatty alcohol profiles obtained from the Y. *lipolytica* strains containing Lbo_PPTQ, HarFAR and different POXes. Cultivation media was supplemented with 0.24% (v/v) of 14:Me.



■ Sum of Z7-12:Me, Z7-14:Me, Z9-14:Me 🛛 12:Me 🔎 14:Me 🔎 16:Me 🔍 Z7-16:Me 🔍 Z9-16:Me 🔍 Z11-16:Me 🔍 Z9-18:Me 🔍 Z9-18:Me 🔍 Z9-18:Me 🔍 Z9-Z12-18:Me

Figure S3. Fatty acid profiles in the form of methyl esters obtained from the *Y*. *lipolytica* strains containing Dmd9, HarFAR and different POXes. Cultivation media was supplemented with 0.24% (v/v) of 14:Me. *: Position of double bond remains to be identified. Error bars represent standard deviations from three technical replicates



Figure S4. Fatty acid profiles in the form of methyl esters obtained from the *Y. lipolytica* strains containing Lbo_PPTQ, HarFAR and different POXes. Cultivation media was supplemented with 0.24% (v/v) of 14:Me. *: Position of double bond remains to be identified. Error bars represent standard deviations from three technical replicates



Figure S5.A. GC-MS chromatograms of Z7-12:OH reference standard and fatty alcohol profiles of ST10313 (red) and ST10384 (blue). B. Mass spectra of Z7-12:OH reference standard (1) and biologically produced Z7-12:OH (2). Cultivation media was supplemented with 0.4% (v/v) of 14:Me.



Figure S6.A. GC-MS chromatograms of Z9-12:OH and Z7-14:OH reference standards together with fatty alcohol profiles of ST10314 (red) and ST10388 (blue). B. Mass spectra of Z9-12:OH reference standard (1) and Z7-14:OH reference standard together with mass spactra of biologically produced Z9-12:OH (3) and Z7-14:OH (4). Cultivation media was supplemented with 0.4% (v/v) of 14:Me.



Figure S7. Growth characteristics and utilization of glycerol and 14:Me by *Y. Lipolytica* strains ST10313, ST10314, ST10384, ST10388 in shake flasks. A. Growth curves during the first 22h in YPG media. B. Dry weight (DW) measurements at 0, 28 and 52h after media exchange. C. Glycerol measurements at 0, 28 and 52h after media exchange. Error bars represent standard deviations. OD measurements were performed in technical dublicates. DW, glycerol and 14:Me measurements were performed in technical triplicates.



Figure S8. Fatty alcohol profiles of the Y. *lipolytica* strains expressing HarFAR-hrGFP-Per2 (ST12410) and HarFAR-hrGFP (ST12413) fusion proteins. ST9138 is the strain that does not have FAR. Error bars represent standard deviations from three technical replicates