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Supplementary Information for

Transforming yeast peroxisomes into microfactories for the efficient production of high-value isoprenoids

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Supplementary Methods.

Chemicals and enzymes. All chemicals were reagent grade. Standards used, such as (*R*)-(+)-limonene (Sigma-Aldrich, 62118), (*S*)-(-)-limonene (Sigma-Aldrich, 62128) and geraniol (Sigma-Aldrich, 48798), were purchased from Merck Life Science A/S (Søborg, Denmark). Isopropyl myristate and dodecane were purchased from PanReac AppliChem. DNA restriction enzymes, USER® Enzyme, Antarctic phosphatase enzyme and T4 DNA ligase were obtained from New England Biolabs (Herlev, Denmark) and used according to the manufacturer's instructions. The proofreading Q5U® Hot Start High-Fidelity DNA Polymerase (New England Biolabs, M0515S) was used for PCR amplification prior to vector construction, and the Phusion High-Fidelity DNA Polymerase (New England Biolabs, M0530S) was used for routine, control PCR amplifications. QIAprep Spin Miniprep Kit (Qiagen ID: 27104) was used for plasmid purification.

Yeast media. Synthetic defined (SD) 2% glucose media was composed of 0.13% (w/v) Yeast Synthetic Drop-out Medium Supplements without histIDI1ne, leucine, tryptophan and uracil (Sigma-Aldrich, Y2001), 0.67% (w/v) Yeast Nitrogen Base w/o AA (Y2025, US Biologicals) and 2% (w/v) D-(+)-glucose monohydrate (16301, Sigma). For galactose-based SD media, glucose was substituted by 2% (w/v) D-(+)-galactose (MG05201, CarboSynth) and 1% (w/v) D-(+)-raffinose pentahydrate (OR06197, CarboSynth). For the slow glucose release SD media, glucose was substituted by 2.5% (w/v) Enpump 200 substrate (Enpresso GmbH) and 0.075% of reagent A (glucose releasing agent). The total available glucose content was experimentally determined to be 20 g/L with a release rate of 0.2 g L^{-1} h^{-1} upon addition of reagent A. 2X SD media refer to SD media with double concentration of each component. Where needed, 5-fluoroorotic acid (FOA; Zymo Research, F9003) was added to SD media at a concentration of 1 mg/mL alongside 0.002% (w/v) histIDI1ne and uracil, 0.004% (w/v) tryptophan and leucine. Plates contained 2% (w/v) bacto-agar without pH adjustment.

Growth conditions. *E. coli* strains were grown aerobically at 37 °C in Luria-Bertani (LB) medium supplemented, when necessary, with ampicillin (100 µg/mL) and/or chloramphenicol (30 µg/ml). Agar (15 g/L) was added prior to sterilization for LB agar plates. Selection of yeast transformants and pre-cultivation were conducted in SD 2% (w/v) glucose solid or liquid media, supplemented with appropriate amino acids. Typical batch cultures for terpene production and quantification were performed in a 100 mL shake flask containing 9 mL of 2-times concentrated

SD galactose media (or SD Enpump media) that was inoculated from a fully-grown overnight culture to reach a starting OD_{600nm}= 0.5. Cells from the overnight pre-culture were washed 2-times with sterile ddH_2O prior to inoculation to remove all traces of glucose. Isopropyl myristate (IPM) was added as an overlay at 10% (v/v) (1 mL) right after inoculation to trap the monoterpenes. For CBGA production, the culture was performed without IPM and supplemented with 0.5 mM olivetolic acid 4 h after inoculation. Cells were then grown for 96 h at 30 °C and 150 rpm shaking. Semi-continuous fed-batch cultures where conducted in an identical manner than batch cultures except that cells were fed every 48 h with 4% (w/v) galactose and 2% (w/v) raffinose and the pH was adjusted to 4.5. The IPM layer was harvested every 48 h to measure monoterpene production.

Transformation procedures. *E. coli Mach1*™ cells were made competent and transformed using the Mix & Go! *E. coli* Transformation Kit (Zymo Research, USA) following the manufacturer procedure. *S. cerevisiae* strains (Supplementary Table 1) were transformed by the lithium acetate method as previously described in (8). After 45 min incubation at 30 ºC and 15 min of heat shock at 42 ºC, cells were plated on SD agar media lacking uracil, leucine, histIDI1ne and tryptophan. Single colonies were chosen from the plate and streaked separately on SD agar plate lacking uracil, leucine, histIDI1ne and tryptophan. The transformants were screened using PCR amplification of the relevant gene(s).

Analytical procedures. The cell concentration was measured by monitoring the optical density (OD) at 600 nm. For quantification of monoterpene, the two-phase cultures were centrifuged at 3900 x g for 10 min, at 20 °C, to separate the IPM and aqueous layers. The IPM top layer was then collected and diluted in hexane accordingly so that the measured concentration would fall in the linear range of the standard curve. Routinely, the IPM overlay was diluted 1:10 in hexane, in the case of regular batch culture, or 1:100 in hexane, in the case of semi-continuous fed-batch culture. The diluted sample was analyzed by GC-FID. For cannabinogerolic acid extraction, cultures where centrifuged at 3900 x g for 10 min, at 20 °C, to separate cells from the media. Pellets were then resuspended in 1 mL water and subsequently extracted with an ethyl acetate and formic acid (0.05% v/v) mixture in a 2:1 ratio. The mixture was vortexed for 10 min with glass beads. The organic phase was separated by centrifugation at 15,000 x g for 10 min. Extraction was repeated three times and the ethyl acetate fractions were pooled, evaporated using a SpinVac and resuspended in methanol prior to LC-MS analysis. GC-FID analysis was performed using an

HP-5MS Ultra Inert column (Agilent Technologies) with a (5%-Phenyl)-methylpolysiloxane stationary phase. Column dimensions were 30 m length × 0.25 mm internal diameter × 0.25 film thickness. Hydrogen was used as a carrier gas at a constant linear velocity of 50 cm s^{-1}. Samples were analyzed utilizing the following oven program: 3 min hold at 40 °C, ramp to 80 °C at a rate of 3 °C/min followed by a ramp to 300°C at a rate of 30°C/min and a 10 min hold. Quantification was carried out by comparison with pure standards. Analysis of *trans*-isopiperitenol and 8-hydroxygeraniol were performed on a Shimadzu 2010 GC-MS using a ZB-5ms column and helium as a carrier gas with a constant velocity of 37 cm s^{-1}. Samples were diluted 1:100 in hexane before injection of 1 µl and analyzed using the following program: 3 min hold at 40 °C, ramp to 80 °C at a rate of 3 °C/min followed by a ramp to 300 °C at a rate of 30 °C/min and a 10 min hold. Qualitative LC–ESI–MS analysis was performed on the Dionex UltiMate® 3000 Quaternary Rapid Separa-tion UHPLC focused system (Thermo Fisher Scientific, Germering, Germany) equipped with a Phenomenex Kinetex XB-C18 column (150 mm × 2.1 mm i.d., 1.7 µm particle size, 100 Å pore size) (Phenomenex, Inc., Tor-rance, CA, USA). The column was operated at 40 °C, and the flow rate was maintained at 0.3 mL/min. The mobile phases were water (A) and acetonitrile (B), both containing 0.05% formic acid. Separations were performed using the following gradient profile: 0 min, 2% B; 1 min, 2% B; 15 min, 98% B; 18 min, 98% B; 19 min, 2% B; 26 min, 2% B. The column outlet was connected to a Bruker Daltonics Compact QqTOF mass spectrometer equipped with electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany). Mass spectra were acquired in negative ion mode, using a capillary voltage of 4500 V, an end plate offset of − 500 V, a drying temperature of 220 °C, a nebulizer pressure of 1.2 bars, and a drying gas flow of 8 L/min. Accurate mass was calibrated using an internal standard of Sodium formate calibrant injected at the beginning of each chromatographic run. All data was analyzed using the Data Analysis 4.3 (Bruker Daltonics) software program.

Supplementary Figures

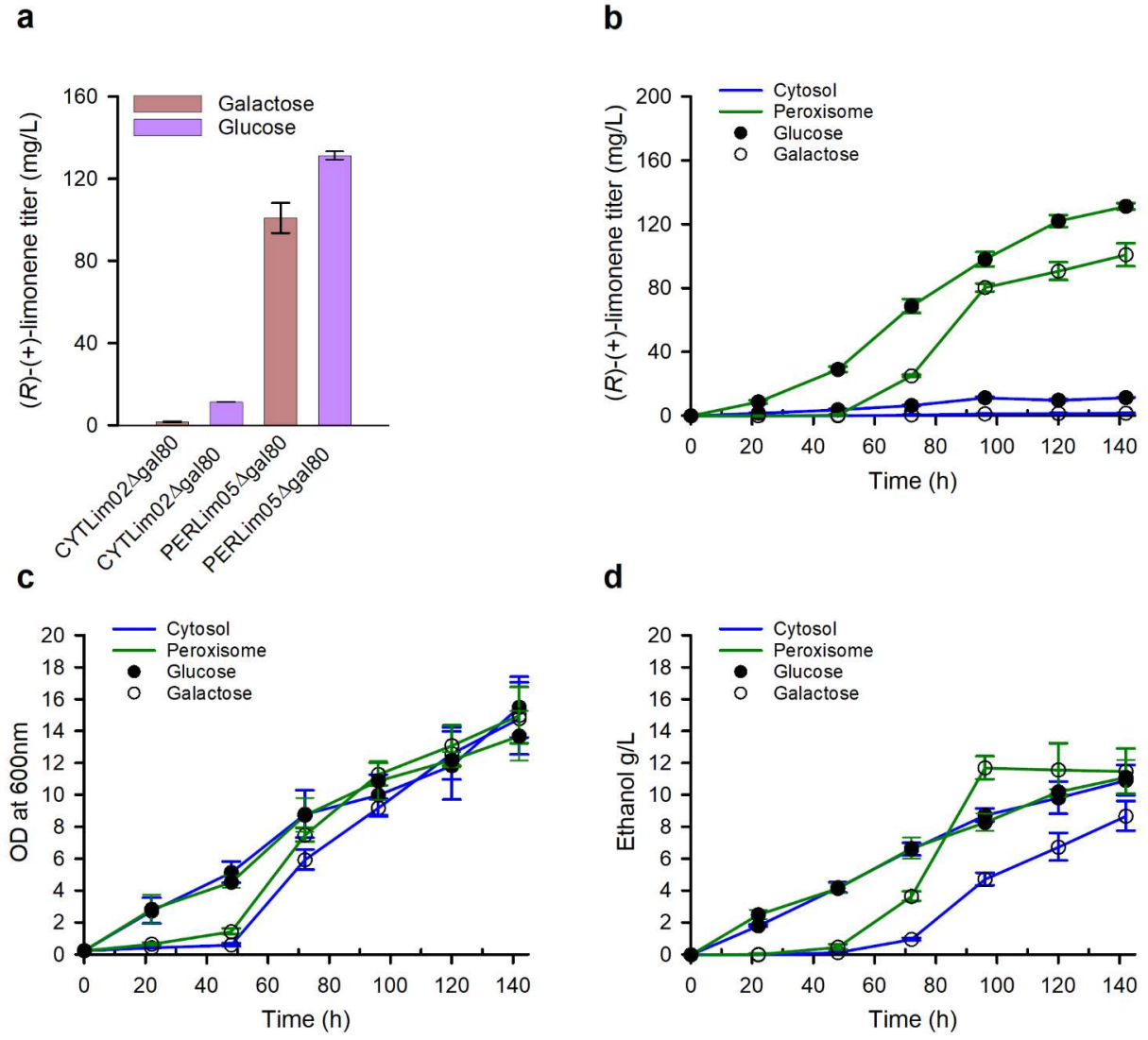

Figure S1. Batch cultures of strains CYTLim02Δgal80 and PERLim05Δgal80. Strains CYTLim02Δgal80 and PERLim05Δgal80 were cultivated in SD-galactose/raffinose (brown) or SD-Enpump (slow glucose release, purple) media and the limonene production was measured after 142 hours (**a**). Time courses of limonene (**b**), biomass (**c**) and ethanol (**d**) accumulation throughout the cultivation with either glucose (closed circle) or galactose (open circle) for the cytosolic pathway (CYTLim02Δgal80, blue line) and for the peroxisomal pathway (PERLim05Δgal80, green line). Error bars correspond to the standard deviation around the mean (n=2, corresponding to two biological replicates).

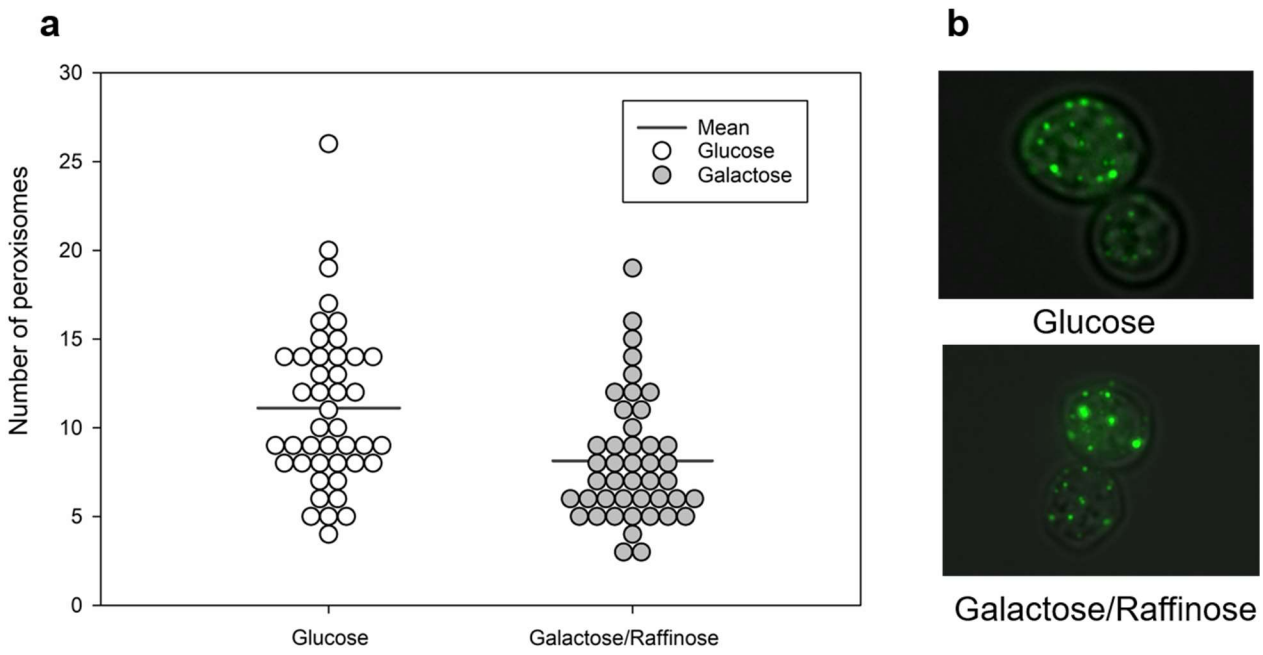

Figure S2. Comparison of the average number of peroxisomes in strain PERGFP grown with glucose or galactose/raffinose as carbon source. Cultures were performed in standard SD 2% (w/v) glucose media or SD 2% (w/v) galactose/1% (w/v) raffinose media. **a.** Peroxisomes were counted using fluorescence microscopy in the strain PERGFP expressing a GFP variant targeted to the peroxisome by fusion of the C-terminal SKL PTS1 tag. In each condition, 50 individual cells were assessed for their peroxisome number, which is then represented by a white circle (glucose condition) or a grey circle (galactose/raffinose) in the graph. The mean number of peroxisomes in each of the two conditions is shown with a gray line. **b.** Typical image for a PERGFP yeast cell grown with glucose as carbon source (top) or galactose/raffinose as carbon source (bottom).

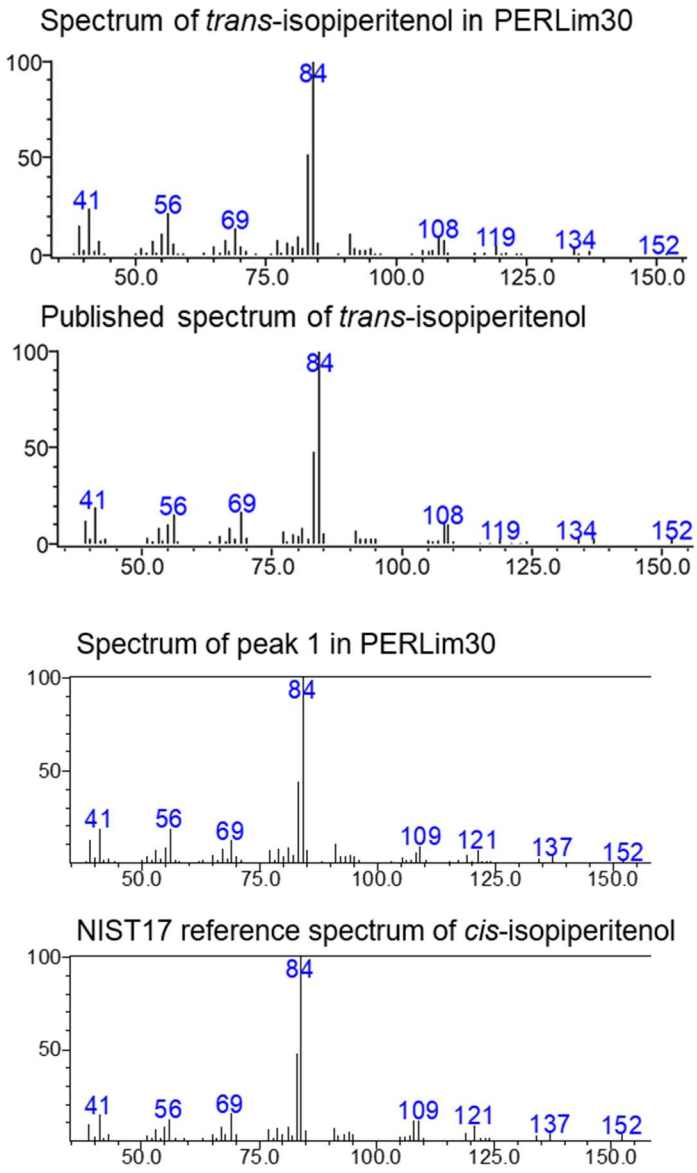

Figure S3. Mass spectrum of *trans*-isopiperitenol and peak 1 produced by PERLim30 cells. Mass spectra of the *trans*-isopiperitenol peak produced by strains PERLim30 and CYTLim06 was obtained by parallel GC-MS analysis of the same samples presented in **Fig. 5a** and identified by comparison to previously reported MS spectra for this molecule (1). Peak labelled 1 (**Fig. 5a**) was tentatively identified as *cis*-isopiperitenol based on its MS spectrum, similarity with available reference spectra, and in agreement with the published product specificity of *Ms*LimH (2).

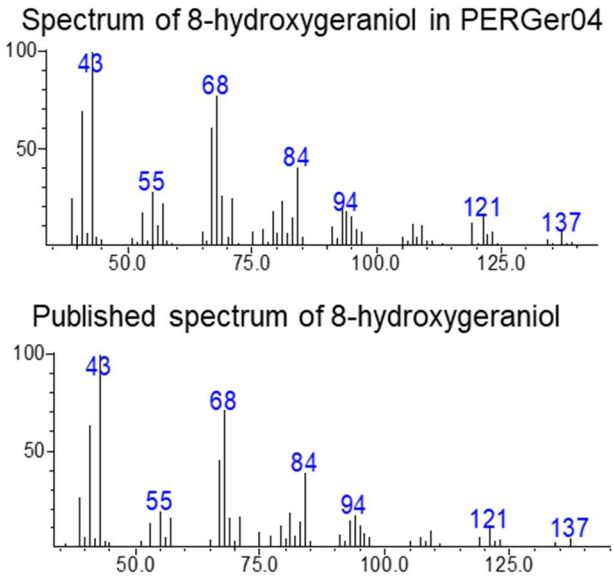

Figure S4. Mass spectrum of the 8-hydroxygeraniol peak produced by PERGer04 cells. Mass spectrum of the 8-hydroxygeraniol peak in sample PERGer04 was obtained by parallel GC-MS analysis of the same samples presented in **Fig. 5b** and confirmed by comparison to previously reported MS spectra for this molecule (3).

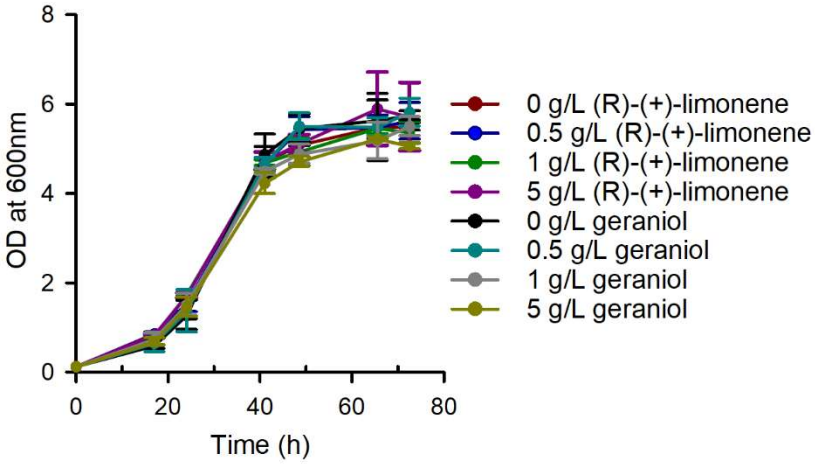

Figure S5. Evaluation of product toxicity. Compared optical density at 600 nm of batch cultures of strain EGY48 with increasing concentration of either (*R*)-(+)-limonene or geraniol added to the 10% IPM overlay to mimic the corresponding monoterpene level observed throughout the semi-continuous fed-batch cultivation of strains PERLim19 and PERGer02. Cultivation was performed in standard SD 2% (w/v) galactose/ 1% (w/v) raffinose media. Error bars correspond to the standard deviation around the mean (n=3, corresponding to three biological replicates).

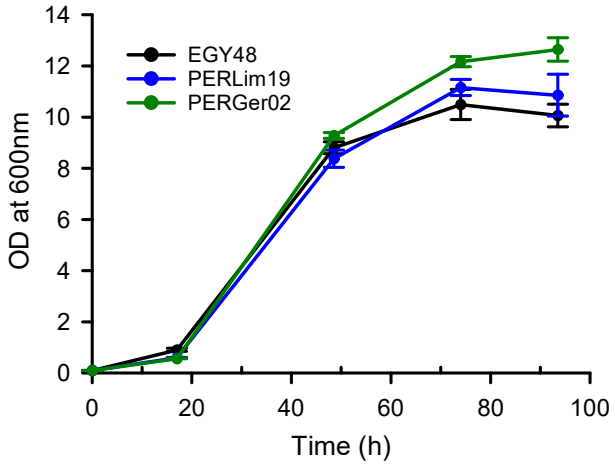

Figure S6. Optical density at 600nm of batch cultures of strains EGY48, PERGer02 and PERLim19. Cultures were grown in standard SD 2% (w/v) glucose media. Error bars correspond to the standard deviation around the mean (n=3, corresponding to three biological replicates).

Supplementary Tables.

Table S1. List of bacterial and yeast strains.

Strain	Plasmid(s) present	Genotype/Relevant characteristics	Source
E. coli Mach1™	-	W ΔrecA1398 endA1 fhuA Φ80Δ(lac)M15 Δ(lac)X74 hsdR(rK–mK+)	Invitrogen (Now: Thermo Fischer Scientific)
EGY48	-	MATα, *ura3, his3, trp1,* 6xLexA operators::LEU2 - Derivative of strain W303-A1	References: (4-6)
EGY48Δgal80	-	EGY48 deleted for the *GAL80* gene	This study
CYTLim01	pCYT08	EGY48 – 2µ; TRP1, HIS3; AmpR; P_{GAL1}-*Cl*LimS-tAHD1, LEU2, URA3	This study
CYTLim02	pCYT01, pCYT02, pCYT03, pCYT04	EGY48–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; P_{GAL1}-*Cl*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTLim02 Δgal80	pCYT01, pCYT02, pCYT03, pCYT04	EGY48Δgal80–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; P_{GAL1}-*Cl*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTLim03	pCYT10	EGY48–2µ; TRP1, HIS3, LEU2, P_{GAL1}-*Ms*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTLim04	pCYT01, pCYT02, pCYT03, pCYT10	EGY48–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; P_{GAL1}-*Ms*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTLim05	-	EGY48 – P_{GAL1}-Efmva-tPGI1, P_{GAL10}-EfmvaE-tCYC1, P_{SED1}-ERG12-tFBA1, P_{Tdh3}-ERG8-tPRM8, P_{Fba1}-ERG19-tSPG5, P_{Cww12}-IDI1-tENO2, P_{Tef1}-ERG20(N127W)-tTDH2, P_{Pgk1}-*Ms*LimS-tADH1.	This study
CYTLim06	pCYT01, pCYT02, pCYT10, pCYT11	CYTLim05–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-*Ms*LimH-tAHD1; P_{GAL10}-*tc*CPR-tCYC1, URA3;	This study

		P_{GAL1}-*Ms*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	
PERLim01	pPER08	EGY48 – 2µ; TRP1, HIS3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1, LEU2, URA3	This study
PERLim02	pCYT01, pCYT02, pCYT03, pPER04	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim03	pCYT01, pCYT02, pPER03, pPER04	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim04	pCYT01, pPER02, pPER03, pPER04	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim05	pPER01, pPER02, pPER03, pPER04	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim05 Δgal80	pPER01, pPER02, pPER03, pPER04	EGY48Δgal80 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim06	pPER04	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim07	pPER03, pPER04	EGY48 – 2µ; TRP1, HIS3, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim08		EGY48 – 2µ; TRP1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-	This study

	pPER02, pPER03, pPER04	IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	
PERLim09	-	EGY48 – P_{GAL1}-EfmvaS-SKL-tPGI1, P_{GAL10}-EfmvaE-SKL-tCYC1, P_{SED1}-ERG12-SKL-tFBA1, P_{Tdh3}-ERG8-SKL-tPRM8, P_{Fba1}-ERG19-SKL-tSPG5, P_{Cww12}-IDI1-SKL-tENO2, P_{Tef1}-ERG20(N127W)-SKL-tTDH2, P_{Pgk1}-*Cl*LimS-SKL-tADH1.	This study
PERLim10	pPER01	PERLim09 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3, LEU2, URA3	This study
PERLim11	pPER02	PERLim09 – 2µ; TRP1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2, URA3	This study
PERLim12	pPER03	PERLim09 – 2µ; TRP1, HIS3, LEU2; AmpR; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3	This study
PERLim13	pPER04	PERLim09 – 2µ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim14	pPER03, pPER04	PERLim09 – 2µ; TRP1, HIS3, LEU2; AmpR; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim15	pPER02, pPER04	PERLim09 – 2µ; TRP1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim16	pPER01, pPER04	PERLim09 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3, LEU2, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim17	pPER02, pPER03, pPER04	PERLim09 – 2µ; TRP1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim18	pPER01, pPER03, pPER04	PERLim09 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1,	This study

		URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	
PERLim19	pPER01, pPER02, pPER03, pPER04	PERLim09 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim20	pPER08	PERLim09 – 2μ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1	This study
PERLim21	pPER11	PERLim09 – 2μ; TRP1, HIS3, LEU2; AmpR; P_{GAL1}-ERG20(N127W)-SKL-tAHD1, URA3	This study
PERLim22	pPER04, pPER09	PERLim09 – 2μ; TRP1, URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1, LEU2	This study
PERLim23	pPER04, pPER10	PERLim09 – 2μ; TRP1, URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1, HIS3; AmpR; P_{GAL1}-ERG12-SKL-tAHD1, LEU2	This study
PERLim24	pPER02, pPER08	PERLim09 – 2μ; TRP1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2, URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1	This study
PERLim25	pPER02, pPER11	PERLim09 – 2μ; TRP1, HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; AmpR; P_{GAL1}-ERG20(N127W)-SKL-tAHD1, URA3	This study
PERLim26	pPER14	EGY48–2μ; TRP1, HIS3, LEU2, P_{GAL1}-*Ms*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim27	pPER01, pPER02, pPER03, pPER14	EGY48–2μ; TRP1, AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Ms*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim28		EGY48 – P_{GAL1}-Efmva-SKL-tPGI1, P_{GAL10}-EfmvaE-SKL-tCYC1, P_{SED1}-ERG12-SKL-tFBA1, P_{Tdh3}-	This study

	-	ERG8-SKL-tPRM8, P_{Fba1}-ERG19-SKL-tSPG5, P_{Cww12}-IDI1-SKL-tENO2, P_{Tef1}-ERG20(N127W)-SKL-tTDH2, P_{Pgk1}-*Ms*LimS-SKL-tADH1.	
PERLim29	pPER01, pPER02, pCfB220, pPER14	PERLim28–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2, URA3; P_{GAL1}-*Ms*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERLim30	pPER01, pPER02, pCYT11, pPER14	PERLim28–2µ; TRP1, AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-*Ms*LimH-tAHD1; P_{GAL10}-*tc*CPR-tCYC1, URA3; P_{GAL1}-*Ms*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
CYTCam01	pCYT07	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Se*CamS -tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTPin01	pCYT05	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; AmpR P_{GAL1}-*Pt*PinS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTSab01	pCYT06	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; AmpR P_{GAL1}-*Sp*SabS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTCam02	pCYT01, pCYT02, pCYT03, pCYT07	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; AmpR; P_{GAL1}-*Se*CamS -tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTPin02	pCYT01, pCYT02, pCYT03, pCYT05	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; AmpR P_{GAL1}-*Pt*PinS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
CYTSab02	pCYT01, pCYT02, pCYT03, pCYT06	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1, HIS3; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1, LEU2; P_{GAL1}-IDI1-tAHD1; $P_{\mathbf{GAL10}}$-ERG19-tCYC1, URA3; AmpR P_{GAL1}-*Sp*SabS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
PERCam01	pPER07	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Se*CamS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study

PERPin01	pPER05	EGY48 – 2μ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Pt*PinS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERSab01	pPER06	EGY48 – 2μ; TRP1, HIS3, LEU2, URA3; AmpR; P_{GAL1}-*Sp*SabS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERCam02	pPER01, pPER02, pPER03, pPER07	EGY48 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; AmpR; P_{GAL1}-*Se*CamS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERPin02	pPER01, pPER02, pPER03, pPER05	EGY48 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; AmpR; P_{GAL1}-*Pt*PinS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERSab02	pPER01, pPER02, pPER03, pPER06	EGY48 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; AmpR; P_{GAL1}-*Sp*SabS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERGer01	-	EGY48 – P_{GAL1}-Efmva-SKL-tPGI1, P_{GAL10}-EfmvaE-SKL-tCYC1, P_{SED1}-ERG12-SKL-tFBA1, P_{Tdh3}-ERG8-SKL-tPRM8, P_{Fba1}-ERG19-SKL-tSPG5, P_{Cww12}-IDI1-SKL-tENO2, P_{Tef1}-ERG20(N127W)-SKL-tTDH2, P_{Pgk1}-ObGerS-SKL-tADH1.	This study
PERGer02	pPER01, pPER02, pPER03, pPER13	PERGer01 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*Ob*GerS-SKL-tAHD1	This study
PERGer03	pPER01, pPER02, pCfB220, pPER13	PERGer01 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2, URA3; P_{GAL1}-*Ob*GerS-SKL-tAHD1	This study
PERGer04	pPER01, pPER02, pCYT12, pPER13	PERGer01 – 2μ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-*cr*G8OH-tAHD1; P_{GAL10}-*cr*CPR-tCYC1, URA3; P_{GAL1}-*Ob*GerS-SKL-tAHD1	This study

PERMva01	pPER01, pPER02, pPER03	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3	This study
PERCan01	pPER15	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; P_{GAL1}-*CsPT4*-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERCan02	pPER01, pPER02, pPER03, pPER15	EGY48 – 2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1, HIS3; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1, LEU2; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1, URA3; P_{GAL1}-*CsPT4*-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
PERGFP	pPERGFP	EGY48 – 2µ; TRP1, HIS3, LEU2, URA3; P_{GAL1}-*GFP*-SKL-tAHD1	This study

Table S2. Comparison of (*R*)-(+)-limonene titers between cytosolic and peroxisomal production in different strains.

Strain	(*R*)-(+)-limonene titer (mg/L)
CYTLim01	0.32 ± 0.01
CYTLim02	1.13 ± 0.05
PERLim01	0.41 ± 0.06
PERLim02	35.68 ± 4.37
PERLim03	43.25 ± 5.73
PERLim04	38.20 ± 0.60
PERLim05	141.46 ± 21.50
PERLim06	3.75 ± 0.47
PERLim07	6.30 ± 0.41
PERLim08	5.79 ± 0.15

Errors correspond to the standard deviation of the mean (n=3, corresponding to three biological replicates).

Table S3. Determining the limiting steps in the peroxisomal pathway. Titers of (*R*)-(+)-limonene obtained in different strains.

Strain	(*R*)-(+)-limonene titer (mg/L)
PERLim09	4.24 ± 1.67
PERLim10	1.96 ± 0.03
PERLim11	3.59 ± 0.04
PERLim12	2.41 ± 0.06
PERLim13	65.79 ± 5.50
PERLim14	71.80 ± 8.03
PERLim15	114.11 ± 5.50
PERLim16	60.31 ± 1.94
PERLim17	106.11 ± 5.41
PERLim18	135.50 ± 12.38
PERLim19	165.89 ± 7.95
PERLim20	32.36 ± 2.08
PERLim21	4.46 ± 0.32
PERLim22	27.92 ± 4.75
PERLim23	116.41 ± 13.74
PERLim24	32.53 ± 3.57
PERLim25	10.74 ± 2.16

Errors correspond to the standard deviation of the mean (n=3, corresponding to three biological replicates).

Table S4. Peroxisomal compartmentalization is a general strategy for monoterpene production in yeast. Titers of monoterpene obtained in different strains and fold improvement compared to cytosolic production.

Monoterpene	Strain	Monoterpene titer (mg/L)	Fold improvement
camphene	CYTCam02	0.39 ± 0.09	
	PERCam02	5.77 ± 1.10	15
sabinene	CYTSab02	1.48 ± 0.17	
	PERSab02	32.32 ± 5.10	22
α-pinene	CYTPin02	0.66 ± 0.02	
	PERPin02	69.22 ± 11.01	105
(*S*)-(-)-limonene	CYTLim04	3.07 ± 0.49	
	PERLim27	51.98 ± 1.29	17
(*R*)-(+)-limonene	CYTLim02	1.13 ± 0.05	
	PERLim05	141.46 ± 21.50	125

Errors correspond to the standard deviation of the mean (n=2, corresponding to two biological replicates).

Table S5. List of episomal plasmids.

Episomal plasmid	Relevant characteristics	Source
pCfB132	pESC-URA-USER	Irina Borodina (7)
pCfB291	pESC-HIS-USER	Irina Borodina (7)
pCfB220	pESC-LEU-USER	Irina Borodina (7)
pWUS	pESC-TRP-USER	This study
pCYT01	2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-tAHD1; P_{GAL10}-EfmvaE-tCYC1	This study
pCYT02	2µ; HIS3; AmpR; P_{GAL1}-ERG8-tAHD1; P_{GAL10}-ERG12-tCYC1	This study
pCYT03	2µ; LEU2; AmpR; P_{GAL1}-IDI1-tAHD1; P_{GAL10}-ERG19-tCYC1	This study
pCYT04	2µ; URA3; AmpR; P_{GAL1}-*Cl*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
pCYT05	2µ; URA3; AmpR P_{GAL1}-*Pt*PinS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
pCYT06	2µ; URA3; AmpR P_{GAL1}-*Sp*SabS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
pCYT07	2µ; URA3; AmpR; P_{GAL1}-*Se*CamS -tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
pCYT08	2µ; HIS3; AmpR; P_{GAL1}-*Cl*LimS-tAHD1	This study
pCYT09	2µ; TRP1; AmpR; P_{GAL1}-LimH-tAHD1; P_{GAL10}-*tc*CPR-tCYC1	This study
pCYT10	2µ; URA3; AmpR; P_{GAL1}-*Ms*LimS-tAHD1; P_{GAL10}-ERG20(N127W)-tCYC1	This study
pCYT11	2µ; LEU2; AmpR; P_{GAL1}-*Ms*LimH-tAHD1; P_{GAL10}-*tc*CPR-tCYC1	This study
pCYT12	2µ; LEU2; AmpR; P_{GAL1}-*cr*G8OH-tAHD1; P_{GAL10}-*cr*CPR-tCYC1	This study
pPER01	2µ; TRP1; AmpR; P_{GAL1}-EfmvaS-SKL-tAHD1; P_{GAL10}-EfmvaE-SKL-tCYC1	This study
pPER02	2µ; HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1; P_{GAL10}-ERG12-SKL-tCYC1	This study
pPER03	2µ; LEU2; AmpR; P_{GAL1}-IDI1-SKL-tAHD1; P_{GAL10}-ERG19-SKL-tCYC1	This study

pPER04	2μ; URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER05	2μ; URA3; AmpR; P_{GAL1}-*Pt*PinS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER06	2μ; URA3; AmpR; P_{GAL1}-*Sp*SabS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER07	2μ; URA3; AmpR; P_{GAL1}-*Se*CamS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER08	2μ; URA3; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1	This study
pPER09	2μ; HIS3; AmpR; P_{GAL1}-ERG8-SKL-tAHD1	This study
pPER10	2μ; HIS3; AmpR; P_{GAL1}-ERG12-SKL-tAHD1	This study
pPER11	2μ; LEU2; AmpR; P_{GAL1}-ERG20(N127W)-SKL-tAHD1	This study
pPER12	2μ; LEU2; AmpR; P_{GAL1}-*Cl*LimS-SKL-tAHD1	This study
pPER13	2μ; URA3; AmpR; P_{GAL1}-*Ob*GerS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER14	2μ; URA3; AmpR; P_{GAL1}-*Ms*LimS-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPER15	2μ; URA3; AmpR; P_{GAL1}-*CsPT4*-SKL-tAHD1; P_{GAL10}-ERG20(N127W)-SKL-tCYC1	This study
pPERGFP	2μ; URA3; AmpR; P_{GAL1}-*GFP*-SKL-tAHD1	This study

Table S6. List of integrative plasmids.

Integrative plasmid	**Relevant characteristics**	**Source**
pAS1_X-3	AmpR; X3-DOWN -DR-URA3-DR-tCYC1-USER cassette* – tPGI1-tFBA1	Victor Forman, (unpublished)
pAS2A	AmpR; tPGI1-tFBA1-USER cassette* – tPRM9-tSPG5	Victor Forman, (unpublished)
pAS2B	AmpR; tPRM9-tSPG5-USER cassette* – tCPS1-tPRM5	Victor Forman, (unpublished)
pAS2C	AmpR; tCPS1-tPRM5-USER cassette* - tENO2-tTDH2	Victor Forman, (unpublished)
pAS3_X-3	AmpR; tENO2-tTDH2-USER cassette* – tADH1- X3-UP	Victor Forman, (unpublished)
pAS_Δgal80	AmpR; gal80-UP -DR-URA3-DR-gal80-DOWN	This study
pIntPER1	AmpR; *URA3*; P_{GAL1}-EfmvaS-SKL-tPGI1; P_{GAL10}-EfmvaE-SKL-tCYC1; X3-DOWN	This study
pIntPER2	AmpR; P_{THD3}-ERG8-SKL-tPRM8; P_{SED1}-ERG12-SKL-tFBA1	This study
pIntPER3	AmpR; P_{FBA1}-ERG19-SKL-tSPG5	This study
pIntPER4	AmpR; P_{CCW12}-IDI1-SKL-tENO2	This study
pIntPER5	AmpR; P_{PGK1}-*Cl*LimS-SKL-tTDH2; P_{TEF1}-ERG20(N127W)-SKL-tADH1; X3-UP	This study
pIntPER6	AmpR; P_{PGK1}-*ObGerS*-SKL-tTDH2; P_{TEF1}-ERG20(N127W)-SKL-tADH1; X3-UP	This study
pIntPER7	AmpR; P_{PGK1}-*MsLimS*-SKL-tTDH2; P_{TEF1}-ERG20(N127W)-SKL-tADH1; X3-UP	

*: USER cassette is constituted of one *Asi*SI restriction site flanked with two *Nb.Bsm*I nicking sites.

Table S7. List of primers used in this study.

Primer	**Sequence (5′-3′)**
GAL1-FP	ACGTATCGCUTTTCAAAAATTCTTACTTTTTTTTTGGAT
GAL1-RP	ACCCGTTGAUGGGTTTTTTCTCCTTGACGTTAAAG
GAL10-RP	ACCCGTTGAUGGGTTTTTTCTCCTTGACGTTAAAGTATA
EfmvaS-FP	ATCAACGGGUAAAATGACCATCGGTATTGACAAAATCTCC
EfmvaS-RP	CGTGCGAUTTAATTTCTGTATGATCTAACTGTGTTATTG
EfmvaS-SKL-RP	CGTGCGAUTTACAGCTTGGAATTTCTGTATGATCTAACTGTGTTATTGATAG
EfmvaE-FP	AGCGATACGUAAAATGAAGACTGTAGTAATAATAGACG
EfmvaE-RP	CACGCGAUTTATTGTTTTCTCAAATCGTTCAAAATTG
EfmvaE-SKL-RP	CACGCGAUTTACAGCTTGGATTGTTTTCTCAAATCGTTCAAAATTGC
ERG8-FP	ATCAACGGGUAAAATGTCAGAGTTGAGAGCCTTCAGT
ERG8-RP	CGTGCGAUTTATTTATCAAGATAAGTTTCCGGATCTT
ERG8-SKL-RP	CGTGCGAUTTACAGCTTGGATTTATCAAGATAAGTTTCCGGATCTTTTTC
ERG12-FP	ATCAACGGGUAAAATGTCATTACCGTTCTTAACTTCTGC
ERG12-RP	CGTGCGAUTTATGAAGTCCATGGTAAATTCGTGTTTC
ERG12-SKL-RP	CACGCGAUTTACAGCTTGGATGAAGTCCATGGTAAATTCGTGTTTC
ERG19-FP	AGCGATACGUAAAATGACCGTTTACACAGCATCCG
ERG19-RP	CACGCGAUTTATTCCTTTGGTAGACCAGTCTTTG
ERG19-SKL-RP	CACGCGAUTTACAGCTTGGATTCCTTTGGTAGACCAGTCTTTG
IDI11-FP	ATCAACGGGUAAAATGACTGCCGACAACAATAGTATGC
IDI11-RP	CGTGCGAUTTATAGCATTCTATGAATTTGCCTGTCATTTTC
IDI11-SKL-RP	CGTGCGAUTTACAGCTTGGATAGCATTCTATGAATTTGCCTGTCATTTTC
*Cl*LimS-FP	ATCAACGGGUAAAATGAGAAGATCAGCTAACTATCAACC
*Cl*LimS-RP	CGTGCGAUTCAACCCTTTGTACCTGGTGATG

*Cl*LimS-SKL-RP	CGTGCGAUTTACAGCTTGGAACCCTTTGTACCTGGTGATGCGGTG
ERG20-FP	ATCAACGGGUAAAATGGCTTCAGAAAAAGAAATTAGGAGAGAG
ERG20-RP	CGTGCGAUCTATTTGCTTCTCTTGTAAACTTTGTTCAAGAAC
ERG20-SKL-FP	CGTGCGAUTTACAGTTTGCTTCTCTTGTAAACTTTGTTC
*Pt*PinS-FP	ATCAACGGGUAAAATGTCATCTACTACATCCGTTTCTAATG
*Pt*PinS-RP	CGTGCGAUTCACAATGGAACGGTTTCAACAACG
*Pt*PinS-SKL-RP	CGTGCGAUTTACAGCTTGGACAATGGAACGGTTTCAACAACGGTTC
*Sp*SabS-FP	ATCAACGGGUAAAATGCGACGCTCTGGGGATTACC
*Sp*SabS-RP	CGTGCGAUTCAGACATAAGGCTGGAATAGCAGG
*Sp*SabS-SKL-RP	CGTGCGAUTTACAGCTTGGAGACATAAGGCTGGAATAGCAGGCC
*Se*CamS-FP	ATCAACGGGUAAAATGTGCAGCCAGCACTCTACTAAAC
*Se*CamS-RP	CGTGCGAUTCATGGAATGGAAACAGCGATGGG
*Se*CamS-SKL-RP	CGTGCGAUTTACAGCTTGGATGGAATGGAAACAGCGATGGGGTC
*Ms*LimH-FP	ATCAACGGGUAAAATGGAATTGCAAATTTCTTCCGCCA
*Ms*LimH-RP	CGTGCGAUTTATGGAGATTTGTACAACGTGGG
*cr*CPR-FP	AGCGATACGUAAAATGGACTCTTCATCTGAAAAGTTGTC
*cr*CPR-RP	CACGCGAUTTACCAGACATCTCTCAAGTATCTAC
*tc*CPR-FP	AGCGATACGUAAAATGCAATCATCAAGCAGCTCGATGA
*tc*CPR-RP	CACGCGAUTTACCATACATCACGCAGATACCTG
*Ms*LimS-FP	ATCAACGGGUAAAATGGAAAGAAGATCCGGTAATTACAATC
*Ms*LimS-RP	CGTGCGAUTTAAGCAAATGGTTCGAACAAAGTTCTGG
*Ms*LimS-SKL-RP	CGTGCGAUTCACAACTTAGACCTTCTACCTCTAC
*Ob*GerS-FP	ATCAACGGGUAAAATGTCTGCTTGTACTCCATTGGC
*Ob*GerS-RP	CGTGCGAUTTATTGGGTGAAGAACAAAGCGTCAAC

*Ob*GerS-SKL-RP	CGTGCGAUTTACAGCTTGGATTGGGTGAAGAACAAAGCG
*Cr*G8OH-FP	ATCAACGGGUAAAATGGACTATTTGACCATTATCTTGACTT
*Cr*G8OH-RP	CGTGCGAUTTACAAGGTAGATGGAACAGCTCTT
*Cs*PT4-FP	ATCAACGGGUAAAATGTCTGCTGGCTCTGACCAAATTG
*Cs*PT4-SKL-RP	CGTGCGAUTTACAGCTTGGAAATAAATACGTAGACGAAATACTCGGC
P_{THD3}-RP	ACCCGTTGAUAGCTTGTGTGTTTATTCGAAACTAAGTTC
P_{SED1}-RP	ACGTATCGCTCTTAATAGAGCGAACGTATTTTATTTTGCTTG
P_{FBA1}-FP	CGTGCGATATGACAGCAGGATTATCGTAATACGTAATAG
P_{FBA1}-RP	ACGTATCGCTGAATATGTATTACTTGGTTATGGTTATATATG
P_{CWW1}-FP	CACGCGATGGATACTTCATGCTATTTATAGACGC
P_{CWW1}-RP	ACCCGTTGATTATTGATATAGTGTTTAAGCGAATGACAGAAG
P_{PGK1}-RP	ACCCGTTGATGTTTTATATTTGTTGTAAAAAGTAGATAATTACTTCC
P_{TEF1}-RP	ACGTATCGCTCTTAGATTAGATTGCTATGCTTTCTTTCTAATG
Gal80-UP-FP	AAGTGCAAGCATAGTGGGGCCTTCTTCCAATGCTAATCCGTCCTCAGCGAGCTCGCATGG
Gal80-DOWN-RP	CTTGATATGAGGATCTAATGGATCAGTTTTTGAAGGCAGCCTAGTCCTGCAGGGGTAACG

Table S8. Nucleotide sequences of the codon optimized genes used in this study.

Name	Sequence (5′-3′)
*Cl*LimS	ATGAGAAGATCAGCTAACTATCAACCATCCATTTGGGACCACGACTTTTTACA ATCCTTGAACTCTAACTACACCGACGAAGCATACAAGAGAAGAGCAGAAGA ATTACGTGGTAAAGTAAAGATAGCCATCAAAGATGTCATCGAACCTTTGGAC CAATTGGAATTGATTGATAACTTGCAAAGATTGGGTTTAGCCCATAGATTTGA AACCGAAATCAGAAACATCTTGAACAACATCTATAACAACAATAAGGATTAC AACTGGAGAAAGGAAAATTTGTACGCTACTTCCTTGGAATTCAGATTGTTAA GACAACACGGTTACCCAGTCAGTCAAGAAGTTTTTAACGGTTTCAAGGATGA CCAAGGTGGTTTTATTTGTGATGACTTCAAGGGTATATTGTCCTTGCATGAAG CTTCTTACTACTCATTGGAAGGTGAAAGTATAATGGAAGAAGCATGGCAATT CACTTCCAAACACTTGAAGGAAGTTATGATAAGTAAAAATATGGAAGAAGAT GTTTTCGTAGCTGAACAAGCAAAGAGAGCCTTGGAATTACCATTGCATTGGA AAGTTCCTATGTTGGAAGCAAGATGGTTCATCCATATATACGAAAGAAGAGA AGATAAGAACCACTTGTTGTTGGAATTGGCAAAGATGGAATTCAATACATTA CAAGCCATCTATCAAGAAGAATTGAAGGAAATCTCTGGTTGGTGGAAGGATA CCGGTTTGGGTGAAAAGTTGTCATTCGCTAGAAATAGATTGGTCGCATCTTTC TTATGGTCAATGGGTATTGCCTTTGAACCTCAATTCGCTTACTGTAGAAGAGT TTTGACAATCTCTATTGCATTGATCACCGTTATAGATGACATCTATGACGTAT ACGGTACTTTGGATGAATTGGAAATCTTTACAGACGCTGTTGAAAGATGGGA TATCAACTATGCATTAAAGCATTTGCCAGGTTACATGAAGATGTGCTTTTTAG CCTTGTACAACTTCGTCAACGAATTCGCTTACTACGTTTTGAAGCAACAAGAT TTCGACTTATTGTTATCTATTAAAAACGCTTGGTTGGGTTTGATACAAGCCTA TTTGGTAGAGGCTAAGTGGTACCATTCTAAGTACACACCTAAGTTGGAAGAA TACTTGGAAAACGGTTTAGTCTCAATCACTGGTCCATTGATCATCACAATCTC CTATTTGAGTGGTACTAACCCTATCATTAAAAAGGAATTGGAATTCTTGGAAT CAAACCCAGATATCGTTCACTGGTCTTCAAAAATTTTCAGATTGCAAGATGAC TTAGGTACTTCCAGTGACGAAATTCAAAGAGGTGACGTACCTAAATCTATAC AATGCTACATGCATGAAACAGGTGCATCAGAAGAAGTTGCCAGACAACACAT TAAGGATATGATGAGACAAATGTGGAAAAAGGTTAATGCTTACACCGCAGAT AAAGACTCCCCATTGACTGGTACTACAACCGAATTCTTGTTGAACTTAGTTAG AATGTCTCATTTCATGTATTTGCATGGTGACGGTCACGGTGTACAAAATCAAG AAACCATTGATGTCGGTTTTACTTTGTTATTCCAACCTATTCCATTAGAAGAT AAACACATGGCATTCACCGCATCACCAGGTACAAAGGGTTGA
*Ms*LimS	ATGGAAAGAAGATCCGGTAATTACAATCCATCAAGATGGGATGTCAACTTCA TCCAGTCTTTGTTGTCCGATTACAAAGAAGATAAGCACGTTATCAGAGCCTCT GAATTGGTTACTTTGGTCAAGATGGAATTGGAGAAAGAAACCGACCAAATCA GACAGTTGGAATTGATTGATGACTTGCAGAGAATGGGTTTGTCCGATCATTTT CAGAACGAGTTCAAAGAGATCCTGTCCTCTATCTACTTGGATCATCATTACTA CAAGAACCCATTTCCAAAAGAAGAGAGGGACTTGTACTCTACTTCTTTGGCTT TCAGACTGTTGAGAGAACATGGTTTTCAAGTTGCCCAAGAAGTTTTCGACTCT TTCAAGAATGAAGAGGGCGAGTTTAAAGAGTCTTTGTCTGACGATACAAGAG GCTTGTTGCAATTATACGAAGCCTCATTCTTGTTGACTGAAGGTGAAACTACT TTGGAATCCGCTAGAGAATTTGCTACCAAGTTCTTGGAAGAAAAGGTTAACG AAGGTGGTGTTGATGGTGATTTGTTGACTAGAATTGCCTACTCCTTGGATATT

	CCATTGCATTGGAGAATCAAAAGACCAAATGCTCCAGTTTGGATCGAGTGGT ATAGAAAAAGACCAGATATGAACCCAGTCGTTTTGGAATTGGCTATCTTGGA TTTGAACATCGTCCAAGCACAATTCCAAGAAGAGTTGAAAGAATCATTCAGA TGGTGGCGTAATACCGGTTTTGTTGAAAAATTGCCATTCGCCAGAGATAGATT GGTTGAATGTTACTTTTGGAACACCGGTATCATCGAACCTAGACAACATGCTT CTGCTAGAATCATGATGGGTAAAGTTAACGCCTTGATCACCGTTATCGATGAT ATCTATGATGTTTACGGCACCTTGGAGGAATTGGAACAATTCACTGATTTGAT CAGAAGGTGGGACATCAACTCTATAGATCAATTGCCAGACTACATGCAGTTG TGTTTTTTGGCCTTGAACAACTTCGTTGATGATACCTCTTACGACGTCATGAA AGAAAAGGGTGTTAACGTTATCCCATACTTGAGACAATCTTGGGTTGATTTGG CTGATAAGTACATGGTTGAAGCTAGATGGTTTTACGGTGGTCATAAGCCATCA TTGGAAGAATACTTGGAAAACTCCTGGCAGTCTATTTCTGGTCCATGTATGTT GACCCATATCTTCTTCAGAGTTACCGACTCCTTTACCAAAGAAACTGTTGACT CCTTGTACAAATACCACGATTTGGTTAGATGGTCCTCATTCGTTTTGAGATTG GCAGATGATTTGGGTACTTCTGTTGAAGAGGTTTCTAGAGGTGATGTTCCAAA GTCCTTGCAATGTTACATGTCTGATTACAACGCTTCTGAAGCTGAAGCAAGAA AACATGTTAAGTGGTTGATTGCCGAAGTCTGGAAAAAGATGAATGCCGAAAG AGTTTCTAAGGACTCTCCATTTGGTAAGGATTTCATTGGTTGTGCTGTTGACTT GGGTAGAATGGCTCAATTGATGTACCATAATGGTGATGGTCATGGTACTCAA CATCCAATTATCCATCAACAGATGACCAGAACTTTGTTCGAACCATTTGCTTA A
*Pt*PinS	ATGTCATCTACTACATCCGTTTCTAATGAAGATGGTGTCCCAAGAAGAATTGC TGGTCATCATTCTAATTTGTGGGATGATGATTCTATCGCCTCTTTGTCTACTTC TTATGAAGCTCCATCTTACAGAAAGAGAGCCGATAAGTTGATTGGTGAAGTC AAGAACATCTTCGACTTGATGTCTGTTGAGGATGGTGTTTTTACTTCTCCATTG TCTGACTTGCATCACAGATTGTGGATGGTTGATTCAGTTGAAAGATTGGGTAT CGACAGACATTTCAAGGACGAAATCAATTCCGCTTTGGATCACGTTTATTCTT ACTGGACCGAAAAAGGTATTGGTAGGGGTAGAGAATCTGGTGTTACTGATTT GAATTCTACCGCTTTGGGTTTGAGAACCTTGAGATTGCATGGTTACACTGTTT CTTCCCACGTTTTGGATCATTTTAAGAACGAAAAGGGTCAGTTCACCTGTTCT GCTATTCAAACTGAAGGTGAAATCAGGGATGTCTTGAATTTGTTCAGAGCTTC CTTGATTGCTTTCCCAGGTGAAAAGATTATGGAAGCTGCTGAAATTTTCTCCA CCATGTACTTGAAAGATGCCTTGCAAAAAATTCCACCATCCGGTTTGTCTCAA GAAATCGAATACTTGTTGGAATTCGGTTGGCATACCAATTTGCCAAGAATGG AAACTAGAATGTACATCGACGTTTTCGGTGAAGATACCACTTTTGAAACCCCA TACTTGATCAGGGAAAAGTTGTTAGAATTGGCCAAGTTGGAGTTCAACATCTT CCATTCATTGGTCAAGAGGGAATTGCAGTCTTTATCTAGGTGGTGGAAAGATT ACGGTTTCCCAGAAATTACCTTCTCCAGACATAGACATGTCGAGTATTATACT TTGGCTGCTTGCATTGCTAACGATCCTAAACATTCTGCTTTCAGATTAGGTTTC GGTAAGATCTCCCATATGATCACCATTTTGGATGATATCTACGACACCTTCGG TACTATGGAAGAATTGAAGTTGTTGACTGCTGCTTTCAAAAGATGGGATCCAT CCTCTATTGAATGCTTGCCAGATTATATGAAGGGTGTTTACATGGCCGTTTAC GACAACATTAACGAAATGGCTAGAGAAGCCCAAAAGATTCAAGGTTGGGAT ACAGTTTCTTACGCTAGAAAATCTTGGGAAGCTTTCATTGGTGCTTACATTCA AGAGGCTAAGTGGATTTCTTCTGGTTACTTGCCAACTTTCGATGAGTACTTGG AAAACGGTAAGGTTTCTTTCGGTTCTAGAATTACTACCTTGGAACCTATGTTG ACCTTGGGTTTTCCATTGCCACCAAGAATATTGCAAGAAATTGACTTCCCCTC

	CAAATTCAACGATTTGATTTGCGCCATTTTGAGGTTGAAGGGTGATACTCAAT GTTACAAAGCTGATAGAGCTAGAGGTGAAGAAGCTTCAGCTGTTTCTTGTTAC ATGAAGGATCATCCAGGTATCACTGAAGAAGATGCCGTTAATCAAGTTAACG CCATGGTTGATAACCTGACCAAAGAGTTGAATTGGGAATTGCTAAGACCAGA TTCAGGTGTTCCAATCTCTTACAAGAAGGTTGCTTTCGATATCTGCAGAGTTT TTCACTACGGTTACAAGTACAGAGATGGTTTCTCTGTTGCTTCCATCGAAATC AAGAACTTGGTTACTAGAACCGTTGTTGAAACCGTTCCATTGTGA
*Sp*SabS	ATGCGACGCTCTGGGGATTACCAACCCTCTCTTTGGGATTTCAATTACATACA GTCTCTCAACACTCCGTATAAGGAGCAGAGATACGTTAATAGGCAAGCAGAG TTGATTATGCAAGTGAGGATGTTGCTTAAGGTAAAGATGGAGGCAATTCAAC AGTTGGAGTTGATTGATGACTTGCAATACCTGGGACTGTCTTATTTCTTTCCA GATGAGATTAAACAAATCTTAAGTTCTATACACAATGAGCACAGATATTTCC ACAATAATGATTTGTATCTCACAGCTCTTGGATTCAGAATCCTCAGACAACAT GGTTTTAATGTTTCCGAAGATGTATTTGATTGTTTCAAGACTGAGAAGTGCAG TGATTTCAATGCAAACCTTGCTCAAGATACGAAGGGAATGTTACAACTTTATG AAGCATCTTTCCTTTTGAGAGAAGGTGAAGATACATTGGAGCTAGCAAGACG ATTTTCCACCAGATCTCTACGAGAAAAACTTGATGAAGATGGTGATGAAATT GATGAAGATCTATCATCGTGGATTCGCCATTCCTTGGATCTTCCTCTTCATTGG AGGATCCAAGGATTAGAGGCAAGATGGTTCTTAGATGCTTATGCGAGGAGGC CGGACATGAATCCACTTATTTTCAAACTCGCCAAACTCAACTTCAATATTGTT CAGGCAACATATCAAGAAGAACTCAAAGATGTCTCAAGGTGGTGGAATAGTT CGTGCCTTGCTGAGAAACTCCCATTTGTGAGAGATAGGATTGTGGAATGCTTC TTTTGGGCCATCGGGGCTTTTGAGCCTCACCAATATAGTTATCAGAGAAAAAT GGCCGCCATTATTATTACTTTCGTAACAATTATCGATGATGTTTATGATGTGT ATGGAACATTAGAAGAACTGGAACTATTTACAGATATGATTCGCAGATGGGA TAATATATCAATAAGCCAACTTCCATATTATATGCAAGTGTGCTATTTGGCAC TATACAACTTCGTTTCTGAGCGGGCTTACGATATTCTAAAAGATCAACATTTC AACAGCATCCCATATTTACAGAGATCGTGGGTAAGTTTGGTTGAAGGATATCT TAAGGAGGCATACTGGTACTACAATGGCTATAAACCAAGCTTGGAAGAATAT CTCAACAACGCCAAGATTTCAATATCGGCTCCTACAATCATATCCCAGCTTTA TTTTACATTAGCAAACTCGACTGATGAAACAGTTATCGAGAGCTTATACGAAT ATCATAACATACTTTACCTATCAGGAACCATATTAAGGCTTGCTGACGATCTT GGGACATCACAACATGAGCTGGAGAGAGGAGACGTCCCGAAAGCAATCCAG TGCTACATGAAGGACACAAATGCTTCGGAGAGAGAGGCGGTGGAACACGTG AAGTTTCTGATAAGGGAGACGTGGAAGGAGATGAACACGGTCACAACAGCC AGCGATTGTCCGTTTACGGATGATTTGGTTGCGGTCGCAACTAATCTTGCAAG GGCGGCTCAGTTTATATATCTCGACGGGGATGGGATTGGCGTGCAACACTCG GAAATACATCAACAGATGGGAGGCCTGCTATTCCAGCCTTATGTCTGA
*Se*CamS	ATGTGCAGCCAGCACTCTACTAAACCATTTAGTCACTCTCCTAATATTTCCAC TAATCTAATTTTATCTTCAGATGGAAGTAATCCTACTAGACGTTCAGGGAATT ACGACCCTACTAAGTGGGATTATGAATATATTCAGTCCGCAAACAATCATTAT ACGGGAGAGAAGTATATGAAGCGATTTAACGAGCTAAAGGCAAAAATTAAG AAAGAATTGATGATGGTTCATGAGGAATCACAAGAATTAGACAAGTTAGAGT TGATTGATAATTTAGAAAGGCTTGGAGTCAGTTACCACTTCAAGGATGAAATT ATGCAAATATTAAGGAGCATTAATGACCAAAGTAATATTGCAGCCACGTCAG

	CAGATTCATTATATTACACTGCCTTGAAATTTAGGATATTGAGGCAACATGGA TTTTATATCTCTCAAGATATACTGAACGATTTCAAGGATGAGAAGGGTAACTT CAAGCAAAGTCTTTGTGAAGACATAGAAGGATTGTTACAATTATATGAAGCA TCATTTCTCTCAACGGATTCTGAAACATCTTCTTTGTTAGAATCCGCCAATAC ATTCGCAACGTCACGTCTAAGGAAATATTTGGACAATTTAAATGGTGATGTTA ATGAGAATTGGAGAGTGGAACTAGTGCGCCATGCCTTGGAACTTCCTTCGCA CTGCATGTTGTTGAGAGTTGAGACAAGATGGTACATTAACATGTATGAGAAA ATACCAAATGCTGATCCTCTTCTGCTCGAGTTTGCTAAATTGGATTTCAATATT GTGCAAGCAACACACCAACAAGAACTAAGAAATCTATCAAGGTGGTGGAAG GAGTCATGGCTTGCAGAAAAATTGTCATTTTCAAGGGACAGAATAGTTGAAT CTTTCTTATGGATAGCTGGCATGATGTTTGAGCCTCAAAGGAACGAACTCTCT CGAACATTGTTAACAAAGGTCACTGCTATCGCGACAATTATAGATGATATTTA TGATGTTTATGGCACTCTTGATGAATTAGAAATCTTCACTCAAGCTGTTGAAA GGATGGAGGTAAAAGCAATGGATGAGCTTCCAGATTATATGAAAGTATGTTA TCTTACACTCATCAATAATACCAATGAAGTGGCGTATGAGGTTCTCAAAGAG CAAGGGATAAATGTCACACCTTACCTTACAAAAGCATGGACAGATTTATGCA AAGCATACTTACAAGAAGCAAGATGGTACTATAGTGGATATACTCCAAGTAT GGAAGAATACATGGAAAATGCATGGGTTTCAGTTGGAAGTCCAGTGATGGTA GTGAATGCATTTTTCTTAGTCACAAATCCAATTACCAAAGAGGCATTGGAACA CCTATTCTTAAACAAGTACACAGAAATAATTCGTTGGTCTGCTGCAATTATTC GTCTCACTGATGACTTAGCCACATCATCTAATGAAATGGAAAGAGGTGATGT TTCCAAGTCGATTCAGTGTTACATGAAAGAGAAGGGCGCTACAGAAGAAGAG GCAAGAAAACATATCAATTTTATGATAAGGGATGCATGGAAACGAATCAACA CAGCTCAAAGAGATAATCCATTATTTTGTGAAGAATTTATTAGCTGTGGAATG AATATTGCAAGAACTGGACAGACCATATACCAGCATGGAGATGGAGTCGGAA TTCAAAATTATGAGATCCAAAATCGCATTTACAAATTATTTTTTGACCCCATC GCTGTTTCCATTCCATGA
*Ob*GerS	ATGTCTGCTTGTACTCCATTGGCTTCAGCTATGCCATTATCTTCTACACCATTG ATTAACGGTGACAACTCCCAAAGAAAGAACACCAGACAACATATGGAAGAG TCCTCTTCAAAGAGAAGGGAATACTTGTTGGAAGAAACCACCAGAAAGTTGC AAAGAAACGATACCGAATCCGTCGAAAAGTTGAAGTTGATCGATAACATCCA ACAGCTAGGTATCGGTTACTACTTTGAAGATGCTATTAACGCCGTTTTGAGGT CCCCATTTTCTACTGGTGAAGAAGATTTGTTTACTGCCGCTTTGAGATTCAGG TTGTTGAGACATAACGGTATCGAAATCTCCCCAGAGATTTTCTTGAAGTTCAA AGACGAAAGAGGTAAGTTCGACGAATCTGATACTTTGGGTTTGTTGTCCTTGT ACGAAGCTTCTAATTTGGGTGTTGCCGGTGAAGAAATATTGGAAGAAGCTAT GGAATTTGCCGAAGCTAGATTGAGAAGATCATTGTCTGAACCAGCTGCTCCA TTGCATGGTGAAGTTGCTCAAGCTTTAGATGTTCCTAGACATTTGAGAATGGC AAGATTGGAAGCCAGAAGATTCATTGAACAATACGGTAAGCAATCCGATCAC GATGGTGATTTGTTGGAATTGGCTATCTTGGATTACAACCAGGTTCAAGCTCA ACACCAATCTGAATTGACCGAAATTATCAGGTGGTGGAAAGAATTGGGTCTA GTTGATAAGTTGTCCTTCGGTAGAGATAGACCATTGGAATGTTTTTTGTGGAC CGTTGGTTTGTTGCCAGAACCTAAATATTCCTCCGTTAGAATTGAATTGGCCA AGGCCATTTCCATCTTGTTGGTTATTGATGATATCTTCGACACCTACGGTGAA ATGGATGATTTGATTTTGTTCACCGATGCCATCAGAAGATGGGACTTAGAAGC AATGGAAGGTTTGCCAGAGTATATGAAGATTTGCTATATGGCCTTGTACAAC ACCACCAACGAAGTTTGTTACAAGGTTTTGAGAGACACCGGTAGAATCGTTTT

	GTTGAACTTGAAGTCTACCTGGATCGATATGATCGAAGGTTTCATGGAAGAG GCTAAGTGGTTTAATGGTGGTTCTGCTCCAAAATTGGAAGAGTACATTGAAA ACGGTGTTTCTACTGCTGGTGCTTATATGGCTTTTGCCCACATATTTTTCTTGA TCGGTGAAGGTGTTACCCACCAAAACTCTCAATTATTCACCCAAAAGCCTTAT CCAAAGGTTTTTTCAGCTGCTGGTAGAATCTTGAGATTGTGGGATGACTTGGG TACTGCTAAAGAAGAACAAGAAAGAGGCGATTTGGCTTCTTGTGTTCAGTTG TTTATGAAGGAAAAGTCCTTGACCGAAGAAGAAGCCAGATCCAGAATTTTGG AAGAGATCAAAGGTTTGTGGCGTGATTTGAATGGTGAATTGGTTTACAACAA GAACCTGCCATTGTCCATTATTAAGGTTGCTTTGAATATGGCCAGAGCCTCTC AAGTTGTTTACAAACATGATCAGGATACCTACTTCTCCTCCGTTGATAATTAT GTTGACGCTTTGTTCTTCACCCAATAA

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