

Applied Microbiology and Biotechnology

Supporting Information

***Saccharomyces cerevisiae* whole cell biotransformation for the production of aldehyde flavours and fragrances**

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A Codon optimised 9-lox from *Nicotiana benthamiana*

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B Codon optimised 9-hpl from *Cucumis melo*

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C GAP promoter sequence from *Pichia pastoris*

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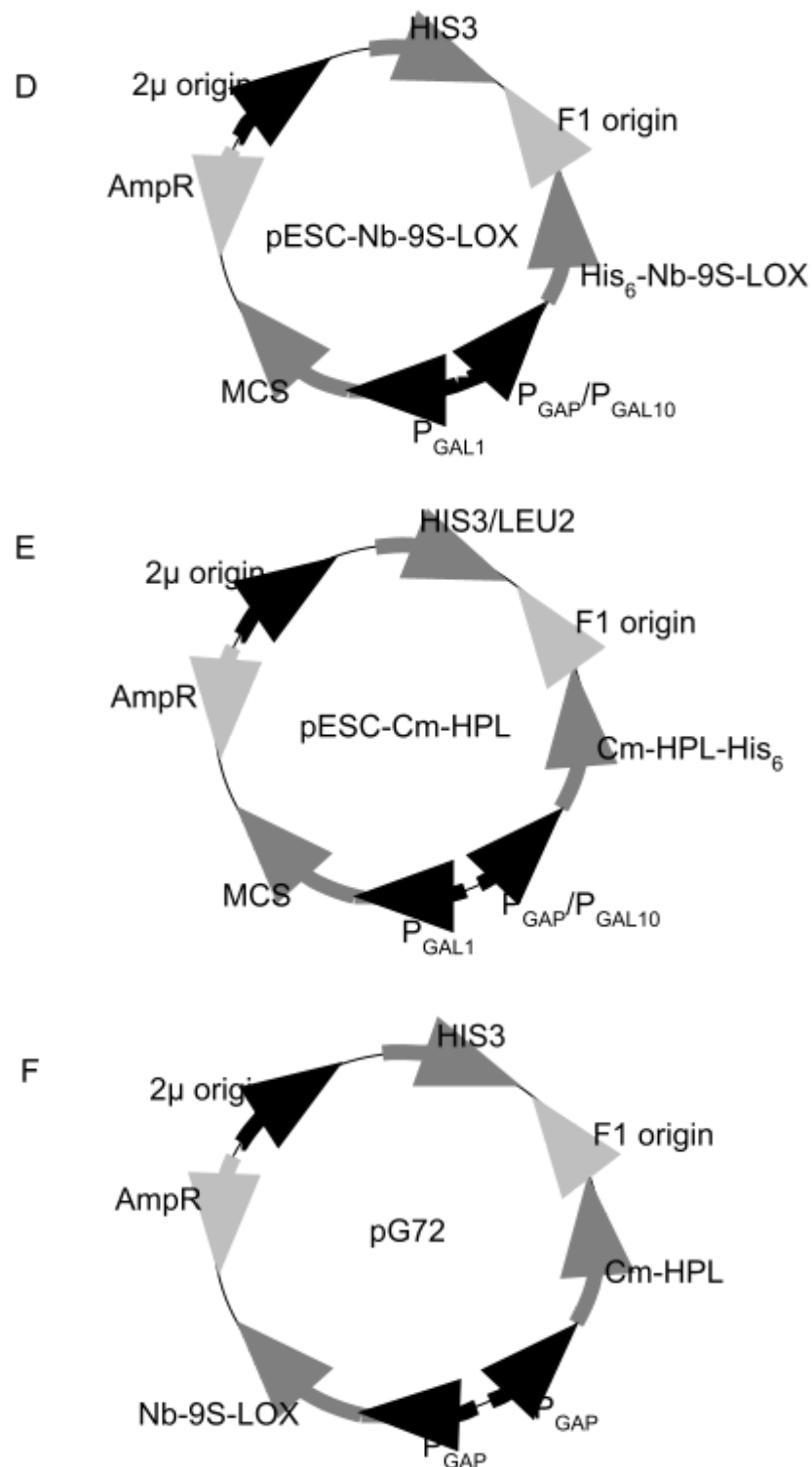


Fig. S1: (A) Codon optimised 9-LOX from *Nicotiana benthamiana* (B) and a 9/13-HPL from *Cucumis melo*. (C) GAP promoter sequence from *Pichia pastoris* (D) pESC-Nb-9S-Lox (E) pESC-Cm-HPL and (F) pG72 plasmids.

Table S1: Primers for construction of plasmids. The underlined sequences indicate the template binding regions

Plasmid	Promoter	Genes	Direction	Primer sequences (5' → 3')
pG72	GAP	Nb-9L ox	Forward	CAATCAATTGAACAACCTATAGGCCTATGTC <u>CTTGGAAAAGATCGTTGATGCC</u>
			Reverse	GTTAGAGCGGATCTTAGCTACTTAGATAGA <u>AACAGAATTAGGGATACCCT</u>
	GAP	Cm-H PL	Forward	TCAATCAATTGAACAACCTATGTCGACATGG <u>CTACTCCATCTTCTTCAT</u>
			Reverse	CCTCTGGCGAAGAATTGTTAATTAAGAGCT <u>CTTAGACCATGTCGGTAGCTCTAG</u>

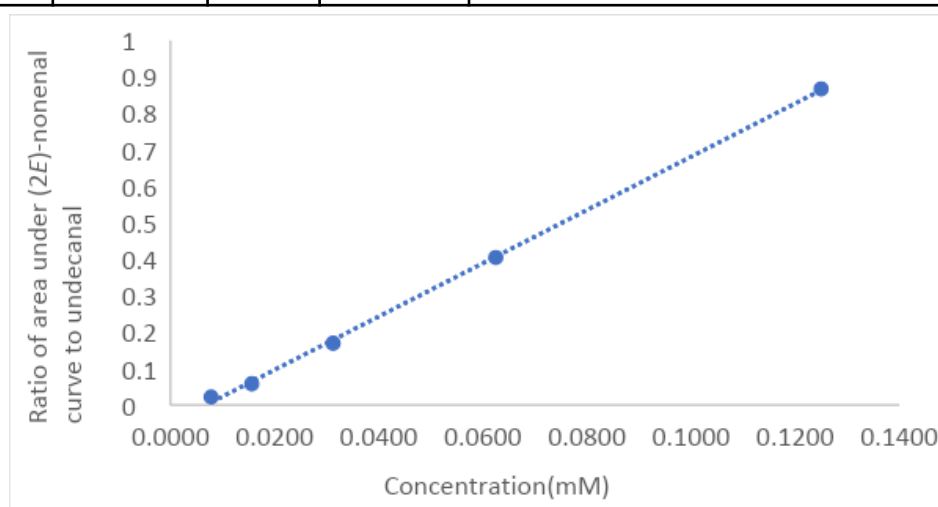


Fig. S2: Calibration curve for (2E)-nonenal. Ratio of area under 5 (2E)-nonenal standard curves to area under respective undecanal curves. A best-fit line was drawn through the points and a standard equation was derived to determine (2E)-nonenal concentration.

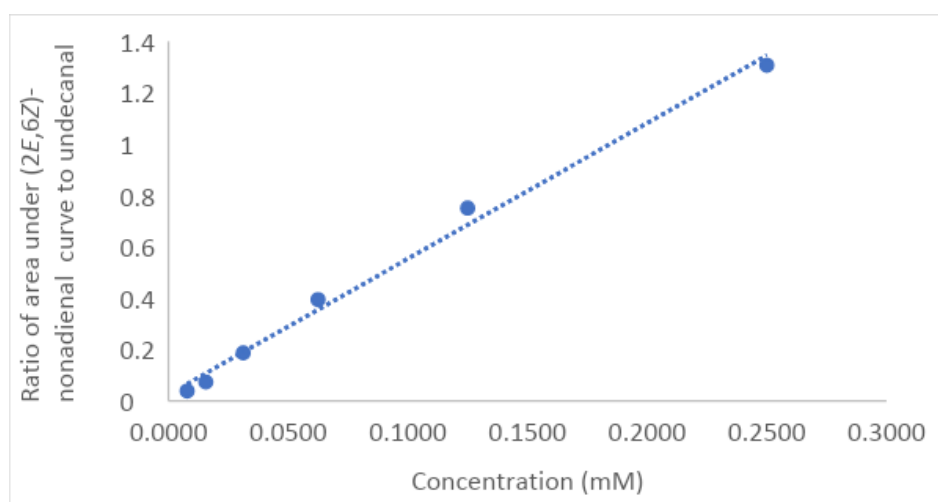


Fig. S3: Calibration curve for (2E,6Z)-nonadienal. Ratio of area under 6 (2E,6Z)-nonadienal standard curves to area under respective undecanal curves. A best-fit line was drawn through the points and a standard equation was derived to determine (2E,6Z)-nonadienal concentration.



Fig. S4: GC-MS analysis chromatogram of G72 biotransformation at (a) 16°C and (b) 30 °C. Permeabilization was performed at OD_{600nm} = 100, with 0.2%(v/v) DMA, for 30 minutes with shaking at 250rpm and at 25°C. Biotransformation reaction was performed with 6mM LA at 16°C or 30 °C for 1 hour, with a cell to substrate ratio of 1.667. Products were detected using GC-MS. Peaks were identified as: 1: hexanal, 3: 2-pentylfuran, 4: (3Z)-nonenal, 6: (2E)-nonenal, 7: (2E)-octenal. Compounds predicted by NIST library include: 15: other nonenal isomer, 16: 2-butyl cyclohexanone.

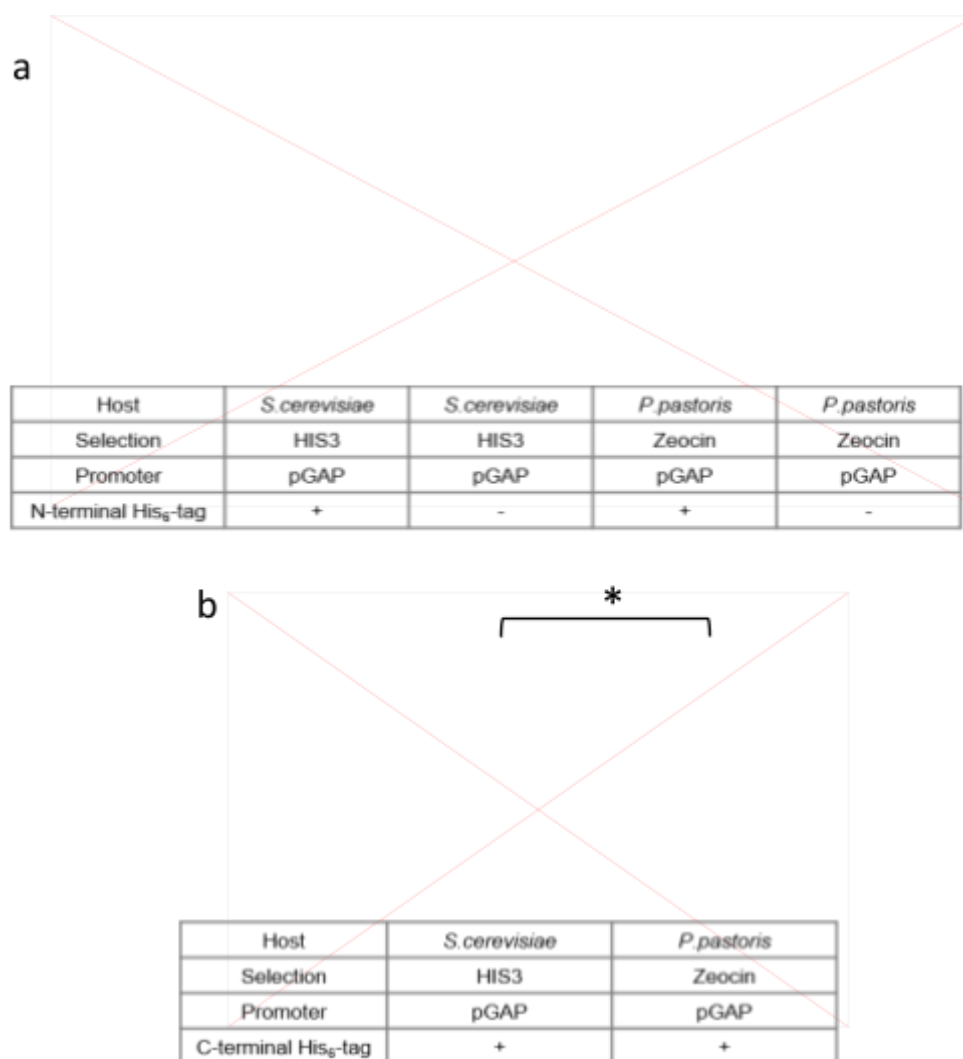


Fig. S5 Activity of LOX and HPL in crude extracts of *S. cerevisiae* and *P. pastoris* as measured by UV assay. Bars represent the average of n=3 biological replicates. Activity of (a) LOX and (b) HPL. X-axis labels indicate host strain, selection marker on the plasmid

(auxotrophic HIS3 for *S. cerevisiae* and 100µg/mL Zeocin (Invitrogen) for *P. pastoris*), promoter driving expression of LOX/HPL (pGAP), presence (+) or absence (-) of a HIS₆ tag attached to LOX/HPL. Error bars represent standard error. Data were analysed using *t*-test: * $P < 0.05$