Supplementary Experiments

6. ESSAYING EFFICIENCY OF THE TOOLKIT

6.1 DNA assemblies

Efficiency of DNA assembly was tested following procedures described in Sections 1.1 – 1.8 of Supplementary Manual. All DNA parts involved in these assemblies are summarised in Table S17. The last column of this table includes the reference for the section where the description of the corresponding assembly type is provided. Resulting efficiencies, estimated as the percentage of correct clones following transformation, are provided in the main text of the manuscript.

6.2 Marker-free *ARO8* and *ARO9* genes disruption.

For marker-free disruption of each, *ARO8* and *ARO9*, gene, three alternative Cas9-helpers with different 20-base pair recognition sequences were constructed: pCasNA-ARO8a, pCasNA-ARO8c, pCasNA-ARO8i and pCasNA-ARO9a, pCasNA-ARO9i, pCasNA-ARO9k, respectively. Two marker-free constructs for *ARO8* and *ARO9* gene disruption were assembled using overlap extension PCR (OE-PCR) with W29 *Y. lipolytica* genomic DNA as the template[1]. For *ARO8* gene, primers were 3204, 3205, 3206, and 3207. For *ARO9* gene primers were 3208, 3209, 3210, and 3211. Each construct represented a fusion of 500 bp of upstream and 500 bp of downstream homology regions flanking the corresponding gene. *Y. lipolytica* W29ΔuraΔku70 strain was co-transformed in the separate with each combination of marker-free construct (donor) and Cas9-helper (Cas9 and guide) according to procedures described in Section 2.2 of Supplementary Manual. Six large colonies from each transformation experiment were analysed by colony PCR using primers pars 3212 and 3213 (for *ARO8* disruption) or 3214 and 3215 (for *ARO9* disruption). Fraction of clones with gene deletion was estimated for each Cas9-helper, including pCasNA-ARO8a (4/6), pCasNA-ARO8c (6/6), pCasNA-ARO8i (5/6) and pCasNA-ARO9a (6/6), pCasNA-ARO9i (4/6), pCasNA-ARO9k (3/6), respectively, with overall efficiency of marker-free gene disruption 77,8% (28/36).

6.3 Marker-free integration of *hrGFP* overexpressing constructs

Three marker-free constructs, pC2S1.1-hrGFP, pD12S1.1-hrGFP, and pE8S1.1-hrGFP were integrated into the genome of *Y. lipolytica* W29ΔuraΔku70 strain as described in Section 2.2 of Supplementary Manual. Integration was verified both by visual assessment of green fluorescence and by colony PCR using standard primers (Tables S5 and S10). As a result, the efficiency of marker-free vector integrations into IntC2, IntD12, and IntE8 loci was estimated as 44.4% (8/18), 88.9% (16/18), and 57.1% (8/14), respectively, with the overall value was equal to 64% (32/50).

7. METABOLIC ENGINEERING

7.1 Engineering HGA producing strain of *Y. lipolytica*

DNA assemblies of plasmid constructs required for metabolic engineering experiments are summarized in Table S17. A pedigree of strains constructed during the metabolic engineering experiment are provided in the flowchart in Figure S29.

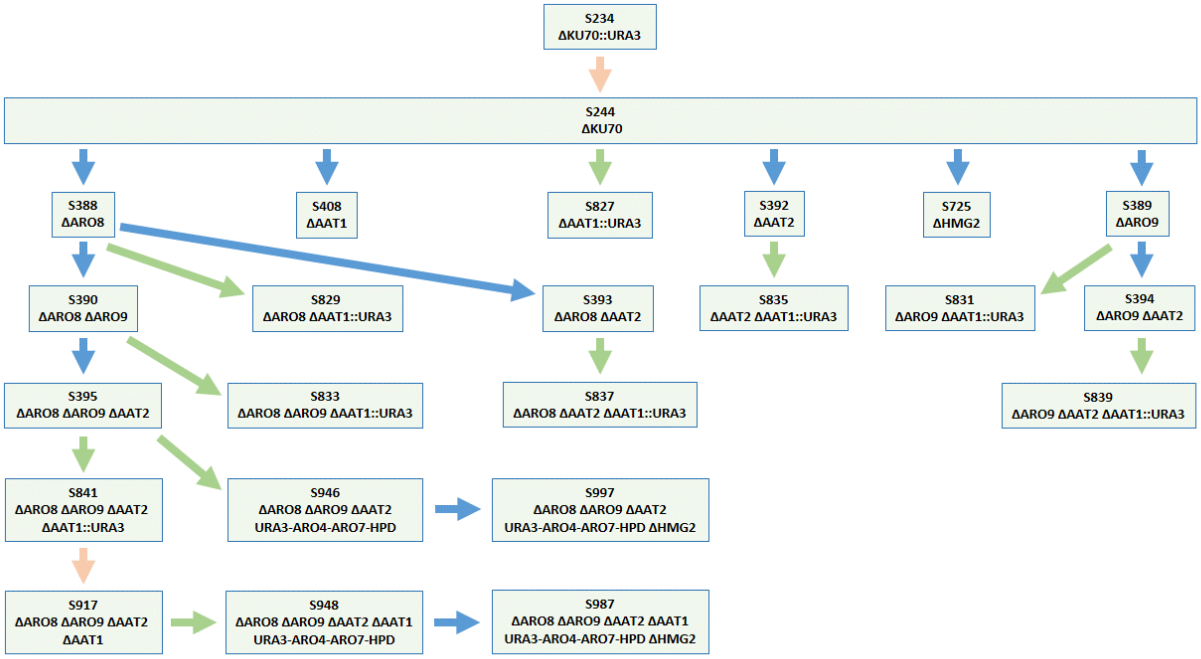

Fig. S29 Pedigree flowchart of the metabolic engineering of HGA producing *Y. lipolytica* strains by combining marker-free and marker-based integration approaches. Blue arrows, marker-free integration steps. Green arrows, marker-based integration steps. Pink arrows, marker recovery steps. All strains contained disruption of *KU70* gene.

Marker-free disruption of *ARO8* and *ARO9* genes was performed as described in Section 6.2. For *AAT2* gene deletion, helper pCasNA-AAT2 was co-transformed with a marker-free disruption construct assembled by OE-PCR using primer set 3296, 3297, 3298, and 3299. Disruption of *AAT2* was verified by colony PCR using primers 3300 and 3301.

AAT1 gene was been disrupted using both marker-free and marker-based approaches. For marker-free approach pCasNA-AAT1 helper was co-transformed with a marker-free disruption construct assembled by OE-PCR using primers set 3308, 3309, 3310, and 3311. The deletion was verified by colony PCR using primers 3312 and 3313. Since initial attempts to disrupt *AAT1* gene in S395 (*Δaro8Δaro9Δaat2*) using the marker-free approach were not successful, we directly compared integration efficiency between S395 and the parental strain S234. Both S395 and S234 were transformed in parallel with the same marker-free construct and pCasNA-AAT1 helper. 8 big colonies were tested from each experiment by colony PCR. None of the tested transformants of strain S395 showed *AAT1* deletions (0/8), while all derivatives of S234 contained the knockout (8/8).

A smilar comparison was done using a marker-based approach. For marker-based *AAT1* gene disruption the construct pDelUK-AAT1 was integrated using procedure described in Section 2.3 of Supplementary Manual. Correct disruptions were verified by colony PCR using primers 3373 and Barcode1-R (Tables S10 and S16). Using this approach, we have isolated *AAT1* gene deletions in S395 with high efficiency (6/12). This confirmed the strict need to use a selectable marker for the introduction of difficult modifications. Moreover, the appearance of transformants (Fig. S30) clearly demonstrated the detrimental phenotype of *Δaro8Δaro9Δaat2Δaat1::URA3* strains. Further comparative analysis using marker-based *AAT1* disruption in strains with different combinations of inactivated aminotransferases clarified that such a detrimental phenotype appeared in all experiments where deletions of *AAT1* and *AAT2* genes were combined (Fig. S31).

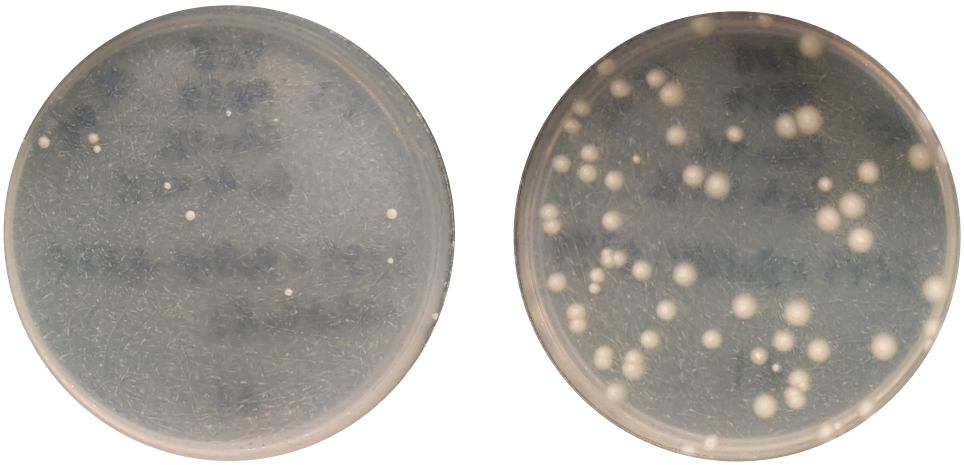

Fig. S30 Detrimental phenotype of *AAT1* gene disruption in strain S395. Both S395 (left) and parental strain W29ΔuraΔku70 (right) were transformed with 500 ng of pDelUK-AAT1 construct under the similar conditions. Transformants were selected on synthetic complete medium (YNB with 1% glucose, 0.4% casamino acids, and 50mM phosphate buffer, pH6.8). Pictures were taken on 9th day of incubation at 30 ^{o}C.

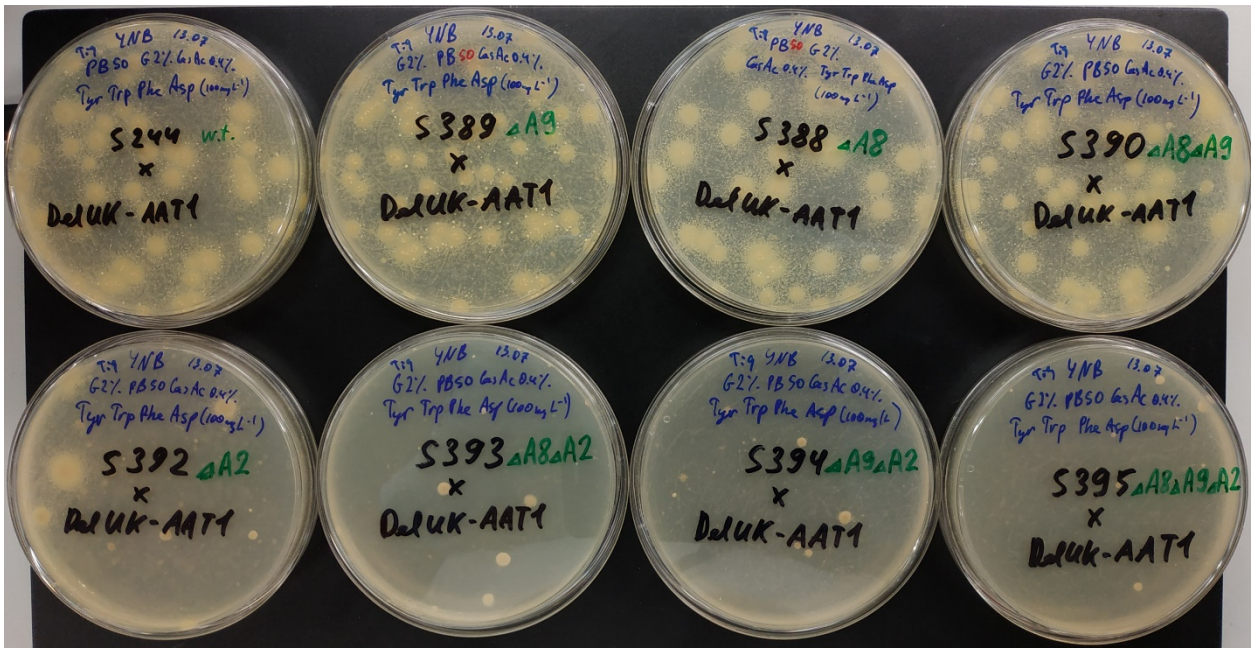

Fig. S31 Detrimental phenotype of combined deletions of *AAT1* and *AAT2* genes. Seven strains with all possible combinations of three aminotransferases gene deletions (*i.e. ARO8, ARO9* and *AAT2*) and parental strain S244 (W29ΔuraΔku70) were transformed with 500 ng of linearized pDelUK-AAT1 under the similar conditions. Transformants were selected on synthetic complete medium (YNB with 2% glucose, 0.4% casamino acids, and 50mM phosphate buffer, pH6.8) with further addition of 100 mg/L of each L-tyrosine, L-tryptophan, L-phenylalanine, and L-aspartate. Plates were imaged after 11 days of incubation at 30 ^{o}C. Detrimental phenotypes were observed in all experiments where recipients with *AAT2* gene disruption were used (bottom row of plates). To check genotypes, please see the pedigree flowchart (Fig. S29).

Thereafter, the marker recovery procedure was applied to remove *URA3* gene from S841 resulting in strain S917 (Section 2.5 of Supplementary Manual). Attempts to transform both lineages (S395 and S917) with a marker-free construct overexpressing three genes (pE8S2.3-HPD-ARO4-ARO7) using pCasNA-IntE8 helper did not lead to observed integration, assessed via colony PCR with primers 3143 and 3171 on 15 transformants from each experiment. Similarly, no integration with the same constructs was observed when parental strain S244 was used as the recipient for transformation (0/15). To verify which gene among those overexpressed lead to the toxic effects halting transformant selection, three transcription units were transformed in S244 separately using marker-free constructs (pE8A1.1-HPD1, pE8A1.2-ARO4 and pE8A1.3-ARO7) targeted to the same IntE8 locus using pCasNA-IntE8 helper. Colony PCR revealed integration of two genes, *HPD1* (5/15) and *ARO7*^{G141S} (7/15), while no correct integrations were observed for *ARO4*^{K229L} (0/15).

To further assess the toxic effect of *ARO4*^{K229L} allele, the random integration vector pZUS1.2-ARO4 was assembled and transformed in recipient stain W29Δura (Table S9), which retained functional NHEJ (Section 2.5 of Supplementary Manual). Random integration approaches frequently lead to integration of the vector parts instead of the whole sequence. Therefore, obtained transformants should be a mixture of clones with either integration of the full-size vector overexpressing *ARO4*^{K229L} or a fragment with the *URA3* marker only. Accordingly, after 11 days of incubation, two clearly distinguishable colony types of different size were observed (Fig. S32). Colony PCR with primers 3064 and 3066 showed that only small colonies contained *ARO4*^{K229L} gene, while large colonies did not give the response, therefore confirming that overexpression of this gene reduced colony viability.

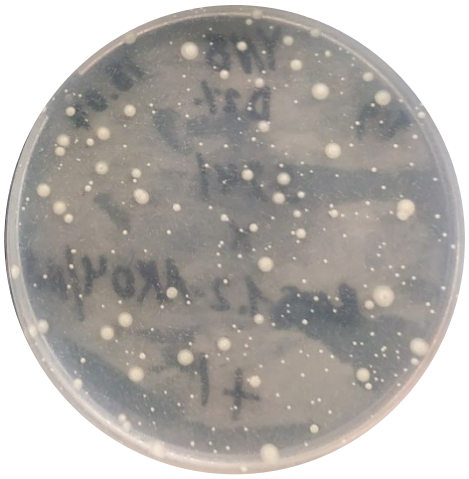

Fig. S32 Detrimental phenotype of *ARO4*^{K229L} overexpression. Plate contains transformants of W29Δura strain with random integrating construct pZUS1.2-ARO4. Picture was taken after 11 days of incubation at 30 ^{o}C on YNB medium with 2% glucose. Only small colonies contained *ARO4*^{K229L} allele.

To assemble both detrimental modifications (namely, double deletion *Δaat2Δaat1* and *ARO4*^{K229L} overexpression) in one strain we decided to combine both selection approaches, *e.g.* to stimulate integration of marker-based construct by inducing double-strand breaks in the target region via Cas9. Indeed, it has been shown that CRISPR/Cas9 assisted integration of marker-based construct yields a significantly higher integration frequency than with the same vector without Cas9 assistance[2]. Both strains, S395 with triple *(Δaro8Δaro9Δaat2*) and S917 with quadruple (*Δaro8Δaro9Δaat2Δaat1*) aminotransferase gene deletions were co-transformed by

pE8US-HPD1-ARO4-ARO7 with pCasNA-IntE8 helper using the two-steps selection procedure (Section 2.4 of Supplementary Manual). Transformants were selected on YNB medium with 2% glucose, casamino acids (5 g/L) and 50mM phosphate buffer (pH6.8). After 10 days of incubation selected clones were analysed by colony PCR using standard primer sets for both flanks of IntE8 locus (Tables S10 and S16). Correct clones were restreaked to single colony on the same medium and presence of all three overexpressed genes, *i.e. HPD1*, $ARO4^{K229L}$, and $ARO7^{G141S}$, was confirmed by colony PCR using primer pairs 3064/3194, 3064/3066 and 3064/3390 respectively. As a result, strains S946 and S948 were obtained as derivatives of S395 and S917 (Fig. S29).

In the final step of engineering the HGA-producing strain, gene *HMG2* was disrupted along with truncation of the telomeric region of chromosome D, as discussed below (Section 7.2). This truncation was induced by a pCasNA-HMG2 helper transformed without application of a donor molecule (Section 2.4 of Supplementary Manual). Transformants of strain lineages with three or four aminotransferase deletions, were recognized by brown halo on YPD medium after 4 days of incubation resulting in derivatives designated as S997 and S987, respectively (Fig. S29).

To check HGA production, YNB media with 9% glucose was inoculated with each strain to an initial OD_{600} of 0.1 in 2.5mL of culture. Three strains were analysed, including both final strains (S997 and S987) and the wild type parent (S234) as the negative control. Cultivation was performed at high aeration rate (300 rpm) at 30^{o} C. After 14 days biomass was separated and HGA concentration was measured in the supernatant by UPLC/MS. Accumulation of HGA by two engineered strains is summarized in Table S11.

Table S11 HGA production by engineered *Y. lipolytica* strains.

Strain number	Strain genotype	HGA production (mg/L)	Standard deviation*
S234	*Δku70::URA3*	**0.0**	0.0
S987	*Δku70Δaro8Δaro9Δaat2Δaat1/↑HPD1↑ARO4↑ARO7/Δhmg2*	**339.1**	11.6
S997	*Δku70Δaro8Δaro9Δaat2/↑HPD1↑ARO4↑ARO7/Δhmg2*	**373.8**	4.1

* – standard deviation was estimated based on two biological replicates

For visual screening of pyomelanin production in liquid media similar cultivation conditions were applied, with a different carbon source and cultivation time. In that case 2% of sodium citrate (pH7.0) was used instead of glucose, with cultivation terminated after 8 days.

7.2 Discovery of *HMG2* gene and putative HGA degradation gene cluster in *Y. lipolytica*

Alignments of the amino acid sequence of homogentisate 1,2-dioxygenase of *Aspergillus fumigatus* (product of *hmgA*, GenBank XM_744461.1) revealed stretches of high similarity in a single region of chromosome D in *Y. lipolytica*. However, both published genomic sequences of W29 strain (GenBank CP028451.1 and CP017556.1) did not reveal an intact open reading frame (ORF) in this region. Consequently, no locus tag was assigned in previous studies for this region during automated genome annotation[3]. Resequencing of this region in W29 strain revealed discrepancies with published sequences and allowed us to identify a new ORF that was designated as *HMG2* (GenBank accession number MZ387986). Analysis of sequences around *HMG2* revealed two genes supposed to be involved in the same pathway of HGA degradation (Fig. S33). Upstream of *HMG2* lies an ORF (ORF2 on the picture) encoding a protein with high level of similarity to a fumarylacetoacetate hydrolase from *A. fumigatus, fahA*. A third ORF next to these two (ORF3 on the picture) encodes putative glutathione S-transferase, the enzyme family to which belongs maleylacetoacetate isomerase encoded by *maiA* gene of *A. fumigatus*. Notably, these three genes of *Y. lipolytica* are placed in the same order and orientation as the HGA-degradation cluster of *Aspergillus sp*[4].

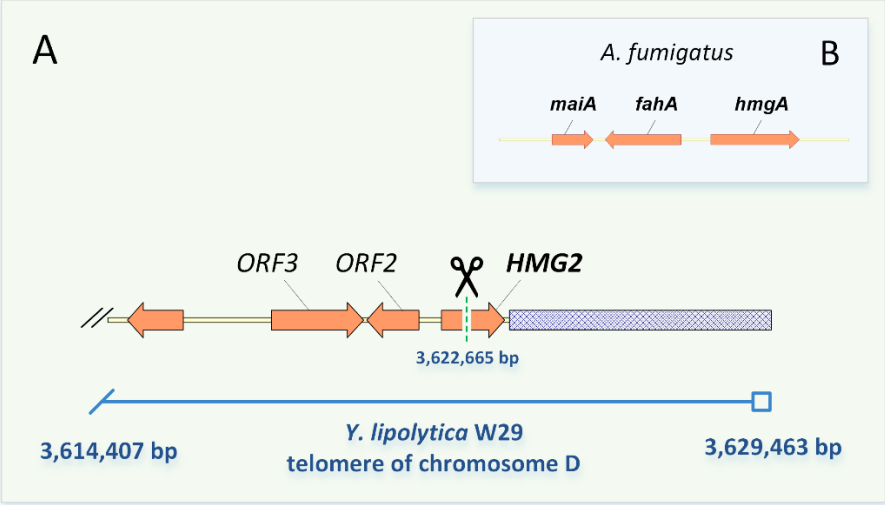

Fig. S33 Putative HGA degradation cluster of *Y. lipolytica*. **A**, structure of the telomeric region of chromosome D of *Y. lipolytica* W29. The gene cluster includes *HMG2* (homogentisate 1,2-dioxygenase), *ORF2* is YALI1_D36215g (putative fumarylacetoacetate hydrolase), and *ORF3* YALI1_D36182g (putative maleylacetoacetate isomerase). Hatched box is a terminal non-unique sequence repeated in the W29 genome. Nucleotide numbering is shown according to the published W29 chromosome D sequence (GenBank CP017556.1). The position of Cas9 cut site is shown that leads to chromosome truncation as described in the text. **B**, structure of similar HGA degradation cluster in *A. fumigatus* (GenBank NC 007195.1).

To test the effect of *HMG2* disruption, we assembled pCasNA-HMG2 helper cutting inside of this gene (Table S17). Since *HMG2* is the last unique sequence on the chromosome D, such a double-strand break will not affect the chromosome integrity if left unrepaired. Surprisingly, transformation of wild type background strain S244 with pCasNA-HMG2 resulted in numerous colonies secreting brown pigment on YPD medium (Fig. S34). It is well known that pyomelanin is the product of self-oxidation and polymerization of HGA formed from aromatic amino acids[5]. At the same time, homogentisate 1,2-dioxygenase depletes HGA and, therefore its inactivation will facilitate pyomelanin formation. Therefore, this disruption enabled phenotype-based characterization of *HMG2* via visualisation of pyomelanin.

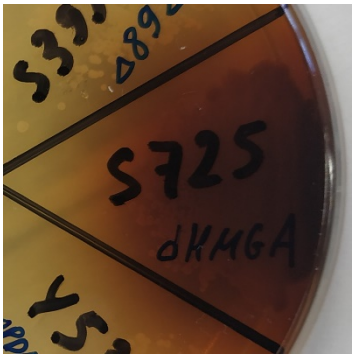

Fig. S34 Bioconversion of aromatic amino acids from rich medium into pyomelanin by *Y. lipolytica* strain with disrupted *HMG2* gene. The strain S725 is S244 derivative with truncation of the telomere region of chromosome D induced by Cas9-helper cutting inside the *HMG2* gene.

We can speculate that truncation of this telomeric region can frequently occur spontaneously in nature. Any double-strand break arising in or upstream of *HMG2* would lead to the characteristic 'brown' phenotype. Due to the chromosomal location of this gene, the cell cycle can proceed without search, bringing together, and repair of the broken DNA ends. This would maintain viability of spontaneous *HMG2* mutants, increasing the frequency of their appearance. Indeed, researchers who work with *Y. lipolytica* often observe mutants over-accumulating brown pigment on rich media[6, 7]. It is noteworthy to mention that the HGA degradation cluster is absent in sequenced genomes of *Y. lipolytica* strains CLIB122 and WSH-Z06, while present in H222 and IBT446. Interestingly, spontaneous pyomelanin accumulation by *Y. lipolytica* induces deep brown coloration of some ripened cheeses (*e.g.* Gorgonzola and Camembert) which leads to significant losses in manufacture[8, 9]. Therefore, a prospective application of this finding might be *Y. lipolytica* strains with a more stable chromosomal location of the HGA degradation gene cluster.

8. PROMOTER LIBRARY SCREENING IN *Y. LIPOLYTICA*

8.1 Natural promoter libraries

A common issue of the toolkits intended for the assembly of multiple transcription units is avoiding repetitive sequences. Application of the same promoter in different positions leads to formation of direct repeats. Recombination between such promoters can induce pop-out of coding sequences locating between them and lead to instability of integrated constructs. To make systems more robust; essential for example for industrial applications; more promoters need to be made available. This is especially true for strong constitutive promoters, such as promoters of *Y. lipolytica* gene *TEF1*, which are limited, while also used very frequently.

As a potential source of promoters with strong constitutive expression we selected genes encoding proteins of ribosomes. Studies on *S. cerevisiae* shown that 50% of the transcripts produced by RNA polymerase II are devoted to the pool of ribosomal proteins[10], while their promoters are among the strongest during exponential growth phase[11]. Therefore, as the source of promoters for the libraries, we selected *Y. lipolytica* genes encoding proteins of small (40S) and large (60S) ribosomal subunits. Commercial synthesis of seven (namely, promoters *RPL5, RPL16, RPL28, RPS5, RPS7, RPS14,* and *RPS30*) was not possible due to complexity of secondary structure and widely distributed homopolymers. As a result, promoters of 26 genes encoding proteins of the small and 38 genes of the large subunit were chosen to test. Besides, we have selected 29 promoters of different origins including one from human cytomegalovirus (*hCMV*) and 28 from *Y. lipolytica*. Among those were genes encoding key metabolic reactions and different house-keeping genes supposed to be highly active in various media and growth conditions. Altogether, three libraries comprised 93 natural promoters that were synthesized and assembled on an empty pProUA-mScarlet vector (Supplementary GenBank files) (Section 1.8 of Supplementary Manual). To make promoters compatible with other modules of the toolkit, we introduced a limited number of point mutations (no more than 4 per promoter) in order to eliminate forbidden recognition sequences (Table S3 of Supplementary Manual).

All obtained pProUA-series plasmids along with *TEF1* promoter (pProUA-TEF1) as a positive control were linearized by MssI and co-transformed with pCasNA-IntC2 helper into the strain S244 (W29ΔuraΔku70) using the procedure described in Section 2.6 of Supplementary Manual. For each construction two independent transformants were isolated and analysed for GFP activity. Parent strain S234 (W29Δku70ura+) was used as a negative control. Strains were first cultivated overnight (16 h) in 2 mL YPD. The biomass was precipitated at 4 ^{o}C, washed with

saline solution and resuspended to get OD_{600} 10.0. At this step, activities of different promoters can be visually compared using blue LED transilluminator (*e.g.* DR46B, Clare Chemical Research) with orange filter (Fig. S35).

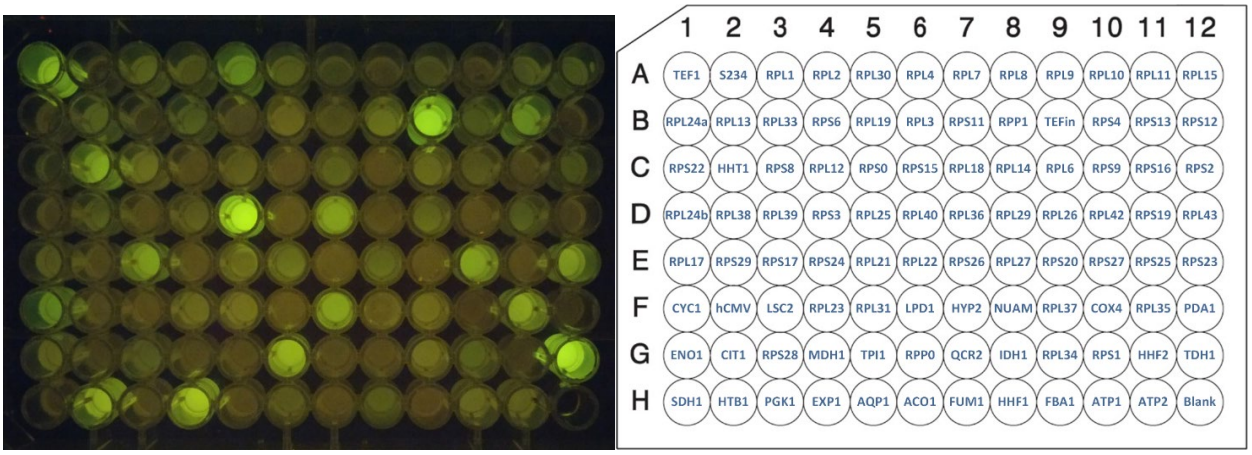

Fig. S35 Visual screening of promoter libraries integrated in *Y. lipolytica* genome using CRISPR/Cas9. Left, three libraries of native promoters were combined in 96-well plate. Single transformant represents each of 96 tested promoters. Right, plate map shown with gene names that were used as the source of the promoters.

Such suspensions were diluted with the same volume of 16% DMSO solution to an OD_{600} 5.0. Then, samples were aliquoted by 10 µL and stored at -80 ^{o}C. For fluorescence measurement these aliquots were defrosted and used to inoculate 2 mL of YPD and YNB media with 2% glucose to a starting OD_{600} of 0.025. Cells were grown at 30 ^{o}C with shaking (250 rpm) until exponential growth phase, which corresponded to 16th and 24th hours of incubation for YPD and YNBD, respectively. Obtained cultures were washed and resuspended in the same volume of saline solution. 200 µl of each suspension was transferred into black-walled 96-well plates with flat transparent bottom (665096 Greiner Bio-One) and green fluorescence (excitation, 495 nm; emission, 535 nm) along with OD_{600} were measured using luminometer CLARIOstar Plus (BMG Labtech). The gain of the photomultiplier was automatically controlled by enhanced dynamic range function. Measured values were normalized for 1 s accumulation time. Acquired data were analysed using MARS software (BMG Labtech). All fluorescence and OD_{600} data were automatically blanked using the reference well with the saline solution. Relative fluorescence (*RF*) for each well was calculated by dividing fluorescence on OD_{600}. Promoter strength (*Px*) was calculated using the following equation:

$$(1)\ P_X(\%) = \frac{RF_{Px} - \bar{X}(RF_{parent})}{\bar{X}(RF_{TEF1} - \bar{X}(RF_{parent}))} \times 100$$

, where RF_{Px}, RF_{TEF1}, and RF_{Parent} are relative fluorescences of strains with promoters *Px*, *TEF1* and the parent strain S234, respectively.

As a result, the autofluorescence of the parent strain S234 (without GFP) was taken as the baseline (0%), while the strength of all promoters was normalized on the *TEF1* activity corresponding to 100%. Overall, during three independent experiments 93 native promoters were functionally characterized (Tables S12, S13, and S14).

Table S12 Measured strength of 26 promoters of *Y. lipolytica* genes encoding 40S ribosomal proteins

Promoter	Source gene	Locus tag	Supposed function of gene product	Donor plasmid	Promoter strength (%)*			
					YPD mean	YPD SD	YNBD mean	YNBD SD
Parent S234					0,0	0,11	0,0	0,54
RPS15	*Y. lipolytica RPS15*	YALI1_F08635g	Ribosomal 40S subunit protein S15	pProUA-RPS15	0,7	0,51	1,32	0,02
RPS26	*Y. lipolytica RPS26*	YALI1_E23610g	Ribosomal 40S subunit protein S26	pProUA-RPS26	1,3	0,35	1,79	0,44
RPS24	*Y. lipolytica RPS24*	YALI1_F32659g	Ribosomal 40S subunit protein S24B	PproUA-RPS24	1,2	0,44	1,96	0,65
RPS20	*Y. lipolytica RPS20*	YALI1_E24442g	Ribosomal 40S subunit protein S20	pProUA-RPS20	2,1	0,37	2,38	1,02
RPS25	*Y. lipolytica RPS25*	YALI1_C05133g	Ribosomal 40S subunit protein S25	pProUA-RPS25	1,7	0,64	3,59	0,98
RPS29	*Y. lipolytica RPS29*	YALI1_B11788g	Ribosomal 40S subunit protein S29	pProUA-RPS29	2,6	1,31	3,81	0,95
RPS16	*Y. lipolytica RPS16*	YALI1_B17008g	Ribosomal 40S subunit protein S16	pProUA-RPS16	4,8	2,2	5,1	0,2
RPS12	*Y. lipolytica RPS12*	YALI1_F09230g	Ribosomal 40S subunit protein S12	pProUA-RPS12	2,1	0,12	6,28	0,98
RPS22	*Y. lipolytica RPS22*	YALI1_D07348g	Ribosomal 40S subunit protein S24A	PproUA-RPS22	7,02	2,3	8,04	0,71
RPS11	*Y. lipolytica RPS11*	YALI1_F27175g	Ribosomal 40S subunit protein S11	pProUA-RPS11	9,7	1,23	12,47	5,54
RPS3	*Y. lipolytica RPS3*	YALI1_E28033g	Ribosomal 40S subunit protein S3	pProUA-RPS3	16,4	0,1	13,14	3,08
RPS8	*Y. lipolytica RPS8*	YALI1_F32305g	Ribosomal 40S subunit protein S8	pProUA-RPS8	22,5	1,3	19,39	1,21
RPS9	*Y. lipolytica RPS9*	YALI1_F08284g	Ribosomal 40S subunit protein S9	pProUA-RPS9	26,6	1,72	19,4	2,61
RPS0	*Y. lipolytica RPS0*	YALI1_A18557g	Ribosomal 40S subunit protein S0	pProUA-RPS0	15,4	0,6	20,76	0,73
RPS4	*Y. lipolytica RPS4*	YALI1_D16029g	Ribosomal 40S subunit protein S4	pProUA-RPS4	28,5	0,3	21,16	4,33
RPS2	*Y. lipolytica RPS2*	YALI1_E17466g	Ribosomal 40S subunit protein S2	pProUA-RPS2	30,4	0,2	22,05	3,63
RPS19	*Y. lipolytica RPS19*	YALI1_B26808g	Ribosomal 40S subunit protein S19	pProUA-RPS19	27,2	0,31	22,96	1,2
RPS28	*Y. lipolytica RPS28*	YALI1_C06597g	Ribosomal 40S subunit protein S28	pProUA-RPS28	28,4	0,14	23,01	4,26
RPP1	*Y. lipolytica RPP1*	YALI1_B16968g	60S acidic ribosomal protein P1	pProUA-RPP1	25,9	0,5	23,3	4,89
RPS1	*Y. lipolytica RPS1*	YALI1_F08431g	Ribosomal 40S subunit protein S1	pProUA-RPS1	25,8	2,7	29,68	2,18
RPS6	*Y. lipolytica RPS6*	YALI1_F25001g	Ribosomal 40S subunit protein S6	pProUA-RPS6	32,1	0,6	30,09	0,24
RPS13	*Y. lipolytica RPS13*	YALI1_F14712g	Ribosomal 40S subunit protein S13	pProUA-RPS13	47,4	4,6	33,8	3,9
RPS17	*Y. lipolytica RPS17*	YALI1_F19249g	Ribosomal 40S subunit protein S17	pProUA-RPS17	42,9	5,66	34,24	1,53
RPS23	*Y. lipolytica RPS23*	YALI1_A21775g	Ribosomal 40S subunit protein S23	pProUA-RPS23	45,4	6,56	35,06	1,58
RPS27	*Y. lipolytica RPS27*	YALI1_E41367g	Ribosomal 40S subunit protein S27	pProUA-RPS27	45,1	5	39,07	2,31
RPP0	*Y. lipolytica RPP0*	YALI1_B18707g	Ribosomal protein P0	pProUA-RPP0	40,6	5,6	41,85	4,68
TEF1	*Y. lipolytica TEF1*	YALI1_C12642g	Translational elongation factor EF-1 alpha	pProUA-TEF1	100	2,33	100	2,89

* - Standard deviation was calculated based on two replicates using independent transformants. Promoter strengths were sorted from weak to strong based on the results obtained in the minimal YNBD medium.

Table S13 Measured strength of 38 promoters of *Y. lipolytica* genes encoding 60S ribosomal proteins

Promoter	Source gene	Locus tag	Supposed function of gene product	Donor plasmid	Promoter strength (%)			
					YPD mean	YPD SD	YNBD mean	YNBD SD
Parent S234					0,0	0,4	0,0	0,33
RPL42	*Y. lipolytica RPL42*	YALI1_B05646g	Ribosomal 60S subunit protein L42	pProUA-RPL42	1,3	1,3	1,95	0,16
RPL10	*Y. lipolytica RPL10*	YALI1_D16249g	Ribosomal 60S subunit protein L10	pProUA-RPL10	3,5	0	2,05	0,27
RPL8	*Y. lipolytica RPL8*	YALI1_F32043g	Ribosomal 60S subunit protein L8	pProUA-RPL8	2,4	0,9	3,37	0,45
RPL39	*Y. lipolytica RPL39*	YALI1_D07338g	Ribosomal 60S subunit protein L39	pProUA-RPL39	1,1	0,3	3,85	0,21
RPL43	*Y. lipolytica RPL43*	YALI1_E41061g	Ribosomal 60S subunit protein L43	pProUA-RPL43	3,8	1,7	5,29	0,75
RPL40	*Y. lipolytica RPL40*	YALI1_F12190g	Ribosomal 60S subunit protein L40	pProUA-RPL40	7,7	3,4	5,52	1,36
RPL24A	*Y. lipolytica RPL24A*	YALI1_E27880g	Ribosomal 60S subunit protein L24A	pProUA-RPL24A	4	2,1	6,07	0,51
RPL19	*Y. lipolytica RPL19*	YALI1_E29930g	Ribosomal 60S subunit protein L19	pProUA-RPL19	5,2	0,5	6,17	0,47
RPL14	*Y. lipolytica RPL14*	YALI1_E00928g	Ribosomal 60S subunit protein L14	pProUA-RPL14	4,1	0,9	6,49	1,02
RPL24B	*Y. lipolytica RPL24B*	YALI1_B13137g	Ribosomal 60S subunit protein L24B	pProUA-RPL24B	4,4	0,4	6,83	2,19
RPL33	*Y. lipolytica RPL33*	YALI1_E37930g	Ribosomal 60S subunit protein L33	pProUA-RPL33	8,8	3,6	7,7	1,43
RPL29	*Y. lipolytica RPL29*	YALI1_F11855g	Ribosomal 60S subunit protein L29	pProUA-RPL29	12,5	0,2	12,94	1,47
RPL9	*Y. lipolytica RPL9*	YALI1_E34344g	Ribosomal 60S subunit protein L9	pProUA-RPL9	19	3,8	12,47	0,6
RPL6	*Y. lipolytica RPL6*	YALI1_E32842g	Ribosomal 60S subunit protein L6	pProUA-RPL6	21,3	1,8	15,85	2,84
RPL11	*Y. lipolytica RPL11*	YALI1_B19897g	Ribosomal 60S subunit protein L11	pProUA-RPL11	24	3,4	16,38	4,77
RPL4	*Y. lipolytica RPL4*	YALI1_C09094g	Ribosomal 60S subunit protein L4	pProUA-RPL4	27,3	0,2	17,08	0,33
RPL17	*Y. lipolytica RPL17*	YALI1_C22922g	Ribosomal 60S subunit protein L17	pProUA-RPL17	22,7	2,7	17,1	3,9
RPL31	*Y. lipolytica RPL31*	YALI1_E29150g	Ribosomal 60S subunit protein L31	pProUA-RPL31	21,4	3,6	17,11	7,36
RPL13	*Y. lipolytica RPL13*	YALI1_B16996g	Ribosomal 60S subunit protein L13	pProUA-RPL13	26,3	1,7	18,07	3,45
RPL2	*Y. lipolytica RPL2*	YALI1_F32090g	Ribosomal 60S subunit protein L2	pProUA-RPL2	21,4	4,1	19,64	2,67
RPL1	*Y. lipolytica RPL1*	YALI1_E37886g	Ribosomal 60S subunit protein L1	pProUA-RPL1	28,9	3,2	20,05	3,93
RPL15	*Y. lipolytica RPL15*	YALI1_D32153g	Ribosomal 60S subunit protein L15	pProUA-RPL15	29,5	2,4	20,37	2
RPL22	*Y. lipolytica RPL22*	YALI1_E38178g	Ribosomal 60S subunit protein L22	pProUA-RPL22	32,6	3,8	20,42	4,34
RPL37	*Y. lipolytica RPL37*	YALI1_F39051g	Ribosomal 60S subunit protein L37	pProUA-RPL37	20,9	1,6	21	2,26
RPL34	*Y. lipolytica RPL34*	YALI1_C06511g	Ribosomal 60S subunit protein L34	pProUA-RPL34	23,6	7,3	21,34	5,2
RPL18	*Y. lipolytica RPL18*	YALI1_B11934g	Ribosomal 60S subunit protein L18	pProUA-RPL18	30,8	1,5	21,59	1
RPL23	*Y. lipolytica RPL23*	YALI1_D12930g	Ribosomal 60S subunit protein L23	pProUA-RPL23	20,1	0,1	22,22	2,38
RPL3	*Y. lipolytica RPL3*	YALI1_C29810g	Ribosomal 60S subunit protein L3	pProUA-RPL3	21,8	1,6	22,46	2,1
RPL27	*Y. lipolytica RPL27*	YALI1_B07937g	Ribosomal 60S subunit protein L27	pProUA-RPL27	35,6	2,8	22,59	0,75
RPL21	*Y. lipolytica RPL21*	YALI1_F08260g	Ribosomal 60S subunit protein L21	pProUA-RPL21	34	0,1	25,77	1,46
RPL7	*Y. lipolytica RPL7*	YALI1_E16649g	Ribosomal 60S subunit protein L7	pProUA-RPL7	18,5	2,6	27,23	0,1
RPL38	*Y. lipolytica RPL38*	YALI1_B14993g	Ribosomal 60S subunit protein L38	pProUA-RPL38	28,8	0,3	26,94	1,64
RPL35	*Y. lipolytica RPL35*	YALI1_A09798g	Ribosomal 60S subunit protein L35	pProUA-RPL35	37,7	1,4	28,38	1,87
RPL12	*Y. lipolytica RPL12*	YALI1_D17206g	Ribosomal 60S subunit protein L12	pProUA-RPL12	34,4	2,9	30	0,06
RPL26	*Y. lipolytica RPL26*	YALI1_E25317g	Ribosomal 60S subunit protein L26	pProUA-RPL26	29,7	2,1	31,23	4,39
RPL36	*Y. lipolytica RPL36*	YALI1_E35963g	Ribosomal 60S subunit protein L36	pProUA-RPL36	36,5	6,2	37,28	1,04
RPL30	*Y. lipolytica RPL30*	YALI1_E27871g	Ribosomal 60S subunit protein L30	pProUA-RPL30	56,3	6,1	48,14	2,49
TEF1	*Y. lipolytica TEF1*	YALI1_C12642g	Translational elongation factor EF-1 alpha	pProUA-TEF1	100	2,33	100	3,73
RPL25	*Y. lipolytica RPL25*	YALI1_F32792g	Ribosomal 60S subunit protein L25	pProUA-RPL25	130,9	9,17	125,66	3,38

Table S14 Measured strength of 29 natural promoters of different sources

Promoter	Source gene	Locus tag	Supposed function of gene product	Donor plasmid	Promoter strength (%)			
					YPD mean	YPD SD	YNBD mean	YNBD SD
Parent S234					0,0	0,33	0,0	0,66
FUM1	*Y. lipolytica FUM1*	YALI0C06776g	Fumarate hydrotase mitochondrial	pProUA-FUM1	0,82	0,52	0,64	0,36
PGK1	*Y. lipolytica PGK1*	YALI1_D15424g	Phosphoglycerate kinase	pProUA-PGK1	0,62	0,27	0,75	0,5
SDH1	*Y. lipolytica SDH1*	YALI1_D14260g	Flavoprotein subunit of succinate dehydrogenase	pProUA-SDH1	0,34	0,31	0,87	0,65
AQP	*Y. lipolytica AQP*	YALI1_F00616g	Protein similar to aquaporin 9 (Small solute channel 1)	pProUA-AQP	0,8	0,7	0,91	0,34
hCMV	*Human Cytomegalovirus*		Human cytomegalovirus (CMV) immediate early promoter	pProUA-hCMV	2,62	0,5	1,72	0,84
LPD1	*Y. lipolytica LPD1*	YALI1_D26360g	Dihydrolipoyl dehydrogenase	pProUA-LPD1	4,08	0,62	2,26	1,38
FBA1	*Y. lipolytica FBA1*	YALI0E26004g	Fructose 1,6-bisphosphate aldolase	pProUA-FBA1	1,36	0,2	2,6	0,33
LSC2	*Y. lipolytica LSC2*	YALI1_D05892g	Beta subunit of mitochondrial succinyl-CoA ligase	pProUA-LSC2	4	0,8	3,95	2,23
NUAM	*Y. lipolytica NUAM*	YALI1_D07089g	Subunit NUAM of NADH:Ubiquinone Oxidoreductase (Complex I)	pProUA-NUAM	4,65	0,32	4,33	1,52
TPI1	*Y. lipolytica TPI1*	YALI1_F07847g	Triosephosphate isomerase	pProUA-TPI1	9,1	1,03	5,94	2,15
COX4	*Y. lipolytica COX4*	YALI1_E23635g	Subunit IV of cytochrome c oxidase	pProUA-COX4	7,4	0,3	6,16	1,79
PDA1	*Y. lipolytica PDA1*	YALI1_F27556g	E1α subunit of the pyruvate dehydrogenase	pProUA-PDA1	6,9	0,27	7,23	2,38
IDH1	*Y. lipolytica IDH1*	YALI1_E06015g	Subunit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase	pProUA-IDH1	11,4	0,81	10,09	3,14
MDH1	*Y. lipolytica MDH1*	YALI1_D20676g	Mitochondrial malate dehydrogenase	pProUA-MDH1	8,98	1,1	10,67	1,09
QCR2	*Y. lipolytica QCR2*	YALI1_F12033g	Subunit 2 of ubiquinol cytochrome-c reductase (Complex III)	pProUA-QCR2	10,55	0,4	10,44	2,48
HHF2	*Y. lipolytica HHF2*	YALI1_F33331g	Histone H4	pProUA-HHF2	8,18	1,5	12,63	0,67
ATP1	*Y. lipolytica ATP1*	YALI1_F04455g	Alpha subunit of the F1 sector of mitochondrial ATP synthase	pProUA-ATP1	19,22	1,24	13,54	3,47
ACO1	*Y. lipolytica ACO1*	YALI1_D11984g	Gene encoding a mitochondrial aconitate hydratase	pProUA-ACO1	18,87	0,6	13,6	1,44
CIT1	*Y. lipolytica CIT1*	YALI1_E03300g	Citrate synthase mitochondrial	pProUA-CIT1	16,33	0,59	17,76	0,56
CYC1	*Y. lipolytica CYC1*	YALI1_D11769g	Cytochrome c	pProUA-CYC1	25,58	3,71	20,06	3,13
ENO1	*Y. lipolytica ENO2*	YALI1_F22436g	Enolase 1	pProUA-ENO1	21,24	1,41	20,11	0,13
HYP2	*Y. lipolytica HYP2*	YALI1_C09230g	Translation elongation factor eIF-5A	pProUA-HYP2	37,24	7,1	27,07	2,84
ATP2	*Y. lipolytica ATP2*	YALI1_B05364g	Beta subunit of the F1 sector of mitochondrial ATP synthase	pProUA-ATP2	22,3	4,4	27,43	2,82
HHF1	*Y. lipolytica HHF1*	YALI1_C15964g	Histone H4	PproUA-HHF1	31,78	0,2	31,49	0,53
HTB1	*Y. lipolytica HTB1*	YALI1_E31389g	Histone H2B	pProUA-HTB1	62,35	1,4	53,85	1,63
HHT1	*Y. lipolytica HHT1*	YALI1_F33345g	Histone H3	pProUA-HHT1	68,9	1,4	61,29	5,75
TEF1	*Y. lipolytica TEF1*	YALI1_C12642g	Translational elongation factor EF-1 alpha	pProUA-TEF1	100	3,7	100	3,33
EXP1	*Y. lipolytica EXP1*	YALI1_C16851g	Cargo receptor protein for Pma1p	pProUA-EXP1	84,76	1,47	103,55	7,02
TEFIn	*Y. lipolytica TEFIn*	YALI1_C12642g	Translational elongation factor EF-1 alpha;	pProUA-TEFIn	117,3	4,8	115	1,81
TDH1	*Y. lipolytica TDH1*	YALI1_C08262g	Glyceraldehyde-3-phosphate dehydrogenase	pProUA-TDH1	122,25	13,38	166,1	12,45

8.2 Hybrid promoter library

The number of natural promoters available for a single organism is limited and their number and properties can be significantly expanded by combining regulatory sequences. Such sequences can be of different origins, including genes derived from other species. Therefore, we decided to use new screening system (*i.e.* the Pro Module) to test another library consisting of hybrid promoters. Initially, we designed 105 promoters. However, only 43 of them were possible to synthesize due to wildly distributed homopolymer sequences. As a result, these 43 hybrid promoters were synthesized and assembled as pProUA-series using commercial service (Supplementary GenBank files). Each promoter was 800 bp in length and combined three to four sequences from seven different yeast species, including *Candida hispaniensis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Komagataella phaffii*, *Ogataea polymorpha, S. cerevisiae,* and *Y. lipolytica* (Section 10). All sequences were selected from upstream regions of genes expected to be highly expressed due to their roles in highly demanded cellular functions, including glycolysis, TCA cycle, pentose phosphate pathway, substrate utilization, cytosolic membrane transport, electron transfer and translation machinery (Table S15).

All pProUA-series plasmids along with a positive control *TEF1* promoter were linearized using MssI and co-transformed with pCasNA-IntC2 into S244 strain (W29ΔuraΔku70) (Section S2.6 of Supplementary Manual). Two independent transformants with each construct were analysed. As the negative control, parental strain S234 (W29Δku70ura+) was used. First, cultures were grown overnight in 2.5 mL of YPD. The biomass was washed with saline solution and diluted in 10% glycerol up to an OD_{600} 1.0. Such seeding material was aliquoted by 10 µL in 96-well flat-bottom plates and stored at -80 ^{o}C. To measure promoter activities on a single-cell level flow cytometry was applied. For this purpose, each aliquot was mixed with 90 µL of either YPG or YNBG, both supplemented with 1.1% glycerol. Therefore, each well contained 100 µL of culture with initial OD_{600} 0.1 and glycerol concentration adjusted to 2%. Microplates were grown in plate reader Synergy HT (BioTek). To increase the aeration plastic lid was used and maximal shaking rate was applied. OD_{600} was measured every 20 min and monitored using Gen5 software (BioTek). Using trial experiments the mid-exponential growth phase was observed at 9th or 15th hours of the incubation in YPG or YNBG media, respectively. At that time point cultivations were stopped and microplates were transferred to flow cytometer Attune NxT (Thermo Fisher Scientific). The fluorescence data were collected from 10,000 cells for each sample and GFP fluorescence was measured using excitation with a 488 nm laser and a 510/10 nm emission filter. The data were analysed using FlowJo software (Ashland, OR). Single value extracted from analysis of each sample was median fluorescence intensity (*MFI*) calculated based on the data collected from the population of individual cells. Promoter strength (*Px*) was estimated using the following equation:

$$(2)\ P_X(\%) = \frac{MFI_{Px} - \underline{X}(MFI_{parent})}{\underline{X}(MFI_{TEF1} - \underline{X}(MFI_{parent}))} \times 100$$

, where MFI_{Px}, MFI_{TEF1}, and MFI_{Parent} are median fluorescence intensity of strains with promoters *Px*, *TEF1* and the parent strain S234, respectively. Resulting strengths of 43 hybrid promoters are summarized in Table S15.

Table S15 Measured strength and composition of 43 hybrid promoters

Promoter	Structure of a hybrid promote				Relative Promoter Strength (%)			
	Source 1 (species gene)	Source 2 (species gene)	Source 3 (species gene)	Source 4 (species gene)	YPG mean	YPG SD	YNBG mean	YNBG SD
Parent S234					0,0	0,01	0,0	0,15
P78	*S. cerevisiae TEF1*	*K. phaffii TEF1*	*S. cerevisiae HXT7*		0,5	0,10	-0,1	0,05
P98	*Y. lipolytica PGK1*	*O. polymorpha CYC1*	*O. polymorpha PSH1*		0,7	0,19	0,1	0,05
P24	*Y. lipolytica GPDH*	*Y. lipolytica ENO2*	*K. marxianus TAL1*		0,4	0,14	0,2	0,30
P1	*S. cerevisiae PFK2*	*O. polymorpha PYK1*	*K. phaffii GND2*		0,0	0,17	0,2	0,50
P36	*S. cerevisiae ZWF1*	*K. phaffii PGK1*	*Y. lipolytica ADH1*		0,5	0,04	0,2	0,05
P55	*Y. lipolytica ENO2*	*O. polymorpha PYK1*	*S. cerevisiae PGK1*		0,8	0,13	0,3	0,10
P11	*S. cerevisiae ENO1*	*K. phaffii GPM1*	*Y. lipolytica GPDH*		0,2	0,08	0,4	0,00
P43	*Y. lipolytica PGK1*	*K. marxianus TAL1*	*K. lactis GPDH*		0,8	0,09	0,4	0,10
P23	*Y. lipolytica TEF1*	*Y. lipolytica ZWF1*	*K. marxianus CYC1*		0,5	0,02	0,4	0,20
P95	*S. cerevisiae TEF1*	*Y. lipolytica ZWF1*	*K. phaffii PYK1*		1,0	0,12	0,4	0,05
P8	*O. polymorpha TPI1*	*O. polymorpha PYK1*	*K. phaffii ADH1*		0,4	0,08	0,9	0,35
P99	*Y. lipolytica GPDH*	*O. polymorpha PFK2*	*K. phaffii AOX1*		0,9	0,03	1,2	0,30
P94	*Y. lipolytica GPDH*	*O. polymorpha PGI1*	*O. polymorpha TAL1*		1,2	0,01	1,4	0,00
P3	*K. phaffii MDH1*	*K. phaffii PFK1*	*K. phaffii GND2*		0,4	0,35	1,4	0,05
P10	*K. phaffii MDH1*	*O. polymorpha ENO2*	*O. polymorpha TAL1*		0,8	0,04	1,4	1,40
P92	*O. polymorpha ZWF1*	*Y. lipolytica PFK1*	*O. polymorpha TAL1*		1,2	0,00	1,5	0,05
P32	*S. cerevisiae ENO1*	*S. cerevisiae ZWF1*	*Y. lipolytica PFK1*		1,2	0,10	1,6	0,05
P13	*Y. lipolytica PGK1*	*K. marxianus MDH1*	*K. phaffii AOX1*		0,5	0,27	1,8	0,00
P37	*Y. lipolytica PGK1*	*Y. lipolytica FBA1*	*Y. lipolytica ENO2*		1,5	0,13	2,1	0,35
P14	*Y. lipolytica GPDH*	*O. polymorpha ENO2*	*K. phaffii AOX1*		1,0	0,03	2,3	0,74
P5	*O. polymorpha ACO1*	*K. phaffii PGK1*	*K. phaffii GND2*		0,5	0,25	2,5	0,05
P50	*Y. lipolytica MDH1*	*K. lactis PFK2*	*K. phaffii AOX*		2,3	0,03	2,5	0,15
P4	*K. phaffii TPI1*	*O. polymorpha ENO2*	*K. phaffii GND2*		0,3	0,26	2,8	0,15
P12	*K. phaffii TPI1*	*S. cerevisiae ADH1*	*Y. lipolytica PFK1*		1,0	0,23	3,2	0,75
P38	*O. polymorpha SDH1*	*O. polymorpha PGI1*	*Y. lipolytica ENO2*		1,8	0,02	3,9	0,30
P2	*K. phaffii ACO1*	*K. phaffii IDP2*	*K. phaffii GND2*		0,4	0,14	4,1	1,20
P73	*S. cerevisiae TEF1*	*K. lactis TEF1*	*K. phaffii AOX1*		5,3	0,17	4,1	0,90
P26	*Y. lipolytica GPDH*	*O. polymorpha PSH1*	*O. polymorpha IDH2*	*Y. lipolytica GPDH*	1,0	0,16	4,9	0,25
P28	*Y. lipolytica TEF1*	*Y. lipolytica ENO2*	*Y. lipolytica PFK1*		2,7	0,13	5,1	0,00
P47	*S. cerevisiae PGK1*	*Y. lipolytica GPDH*	*S. cerevisiae PGK1*	*K. phaffii ADH1*	0,9	0,08	5,8	1,00
P103	*K. lactis ENO1*	*Y. lipolytica ENO2*	*Y. lipolytica GPDH*		4,9	0,03	5,9	0,55
P101	*Y. lipolytica PGK1*	*Y. lipolytica ZWF1*	*Y. lipolytica GND2*		4,7	0,36	6,8	0,20
P96	*K. phaffii TPI1*	*Y. lipolytica TPI1*	*Y. lipolytica GND2*		5,1	0,40	8,1	0,51
P34	*Y. lipolytica STL1*	*K. marxianus MDH1*	*Y. lipolytica GND2*		5,5	0,46	12,9	0,67
P25	*S. cerevisiae PFK2*	*S. cerevisiae ZWF1*	*Y. lipolytica FBA1*		3,9	0,28	13,8	0,78
P97	*K. phaffii PMA1*	*K. phaffii PGK1*	*Y. lipolytica FBA1*		5,8	0,51	14,3	0,25
P45	*Y. lipolytica ENO2*	*Y. lipolytica GPDH*	*Y. lipolytica ACO1*	*Y. lipolytica ENO2*	6,1	0,59	15,2	0,72
P46	*K. phaffii CYC1*	*Y. lipolytica GPDH*	*Y. lipolytica ZWF1*	*K. phaffii CYC1*	7,9	0,93	18,2	0,10
TEF1					100,0	6,32	100,0	23,14
P68	*S. cerevisiae TEF1*	*C. hispaniensis TEF1*	*Y. lipolytica TEF1*		133,5	24,43	137,2	2,94
P67	*Y. lipolytica TEF1*	*Y. lipolytica TEF1*	*C. hispaniensis TEF1*	*Y. lipolytica TEF1*	106,1	2,32	143,0	8,03
P88	*Y. lipolytica TEF1*	*K. marxianus TEF1*	*Y. lipolytica TEF1*		119,0	18,97	156,2	0,91
P89	*S. cerevisiae TEF1*	*K. marxianus TEF1*	*Y. lipolytica TEF1*		134,5	13,66	168,0	1,99
P62	*S. cerevisiae TEF1*	*K. lactis TEF1*	*Y. lipolytica TEF1*		130,1	3,78	174,7	0,78

9. CHARACTERISATION OF STANDARD INTEGRATION LOCI

For each of 16 standard integration loci a Lvl1.1 plasmid was constructed with promoter *TEF1*, gene *hrGFP*, and terminator *LIP2* (Table S17). Obtained overexpression constructs (pB8US1.1-hrGFP, pB11US1.1-hrGFP, pC2US1.1-hrGFP, pC7US1.1-hrGFP, pC13US1.1-hrGFP, pC14US1.1-hrGFP, pD6US1.1-hrGFP, pD12US1.1-hrGFP, pE6US1.1-hrGFP, pE8US1.1-hrGFP, pE12US1.1-hrGFP, pE15US1.1-hrGFP, pE16US1.1-hrGFP, pF8US1.1-hrGFP, pF9US1.1-hrGFP, and pF11US1.1-hrGFP) were linearized and co-transformed together with corresponding standard Cas9-helpers (pCasNA-IntB8, pCasNA-IntB11, pCasNA-IntC2, pCasNA-IntC7, pCasNA-IntC13, pCasNA-IntC14, pCasNA-IntD6, pCasNA-IntD12, pCasNA-IntE6, pCasNA-IntE8, pCasNA-IntE12, pCasNA-IntE15, pCasNA-IntE16, pCasNA-IntF8, pCasNA-IntF9, and pCasNA-IntF11) into S244 (W29ΔuraΔku70) strain of *Y. lipolytica* (Section 2.6 of Supplementary Manual). Successful integration for each locus was confirmed using colony PCR with standard primer sets (Tables S5 and S10 of Supplementary Manual). Efficiency of Cas9-mediated integration for 16 standard loci are summarized in Table S5 of Supplementary Manual. Two independent transformants were isolated for each locus and hrGFP fluorescence was assayed in plate reader as described in Section 8.1. Parent strain S234 (W29Δku70ura+) was used as the negative control. The relative expression levels in 16 loci are compared in Figure S27 and discussed in Section 2.1 of Supplementary Manual.

10. APPENDIX

Table S16 Oligonucleotides used in this study (*continued on the next page*)

#	Name	Sequence*
3193	HPD-F	gcatCGTCTCATCGGGGTCTCAAATGTCACCTTCCGTCGAAGTC
3194	HPD-OE-R	CATGGGTGGTCACATCTCGAGAGCCAGTCTCAAG
3195	HPD-OE-F	GCTCTCGAGATGTGACCACCCATGTCGTGGGC
3196	HPD-R	ctgaCGTCTCTGGTCGGTCTCATAGATTAAAGGTTGCCTCGCTTGGCCTG
3459	ALK1-F	gcatGAAGACTCACGGACTGACTTGATACGCAACTG
3460	ALK1-R	atgcGAAGACAGCATTGTGCAGGAGTATTCTGGGGAG
3369	AAT1-Up950-F	atgcGCTCTTCACAGGTTTAAACGCGTCTTTAACAGGCGAAAAAC
3370	AAT1-Up950-R	gataGCTCTTCTTGGCCTCCTTCTCGGCCTCTC
3371	AAT1-Dn950-F	atgcGCTCTTCATAATGTCGAGCAGGGCCACGAG
3372	AAT1-Dn950-R	gataGCTCTTCTTCCGTTTAAACTGTCCTACAGTTTACACAC
3373	AAT1-chr-F2	GTGCGCACTCTCTCACACC
3204	ARO8-Up500-F	CCAGATTTATATACCGAACACC
3205	ARO8-Up500-R	ACTTTTCCGCCTCTTTGTAGTCGTGCTTTTGG
3206	ARO8-Dn500-F	ACGACTACAAAGAGGCGGAAAAGTCTCTGCTC
3207	ARO8-Dn500-R	TCTCTCTCCCACATGTATGG
3212	ARO8-chr-F	CATACAAACATTCCATGTCGC
3213	ARO8-chr-R	GGCCTGTTTCTTGCATTACTC
3208	ARO9-Up500-F	TTTGGTGACGGAATAAGTCTC
3209	ARO9-Up500-R	TACAGAAAACACCGCTGGAAACAGTGATGATATAG
3210	ARO9-Dn500-F	CTGTTTCCAGCGGTGTTTTCTGTATAGTACAAG
3211	ARO9-Dn500-R	ATTGAGTGGCAATTCTGAACC
3214	ARO9-chr-F	GGTTACCTTATCATGCATGTG
3215	ARO9-chr-R	ACGTCTCAAAAGTGTCCAAAC
3308	AAT1-Up500-F	AACCAACCAAATAACCAAATAAC
3309	AAT1-Up500-R	AATCTGAGAGGTGTTTTGAGAGTCTGGTGGAG
3310	AAT1-Dn500-F	AGACTCTCAAAACACCTCTCAGATTGGTATGTTC
3311	AAT1-Dn500-R	GATGCTCGGTTACAGTCTAC
3312	AAT1-chr-F	CTAGTGGTCGACGACAACC
3313	AAT1-chr-R	GTAGTAAACAATAGCTAGTCAG
3296	AAT2-Up500-F	CTCCACCATCTAGTCATCTC
3297	AAT2-Up500-R	CGCATCTCGCAAGGCCGAAAAGGGCATCTG
3298	AAT2-Dn500-F	CTTTTCGGCCTTGCGAGATGCGCTCAAGAG

3299	AAT2-Dn500-R	TCGTGTCTACAAGTTGCTGC
3300	AAT2-chr-F	CCACGGATCCGCTGAAGC
3301	AAT2-chr-R	CCATACTAACAACTAAGCTCTC
3064	TEF-seq-F1	CTGCAGTCTGGAATCTACGC
3066	ARO4-seq-R	GGTTTCTCCAGATAAGCTCTC
3390	ARO7-seq-R	CTCGTCGGGAGACTCGAAG
3149	Rdm1-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**ATATTATTGTACACCTACCG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3150	Rdm2-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GCATCAGGTGGACTAGCATG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3151	Rdm3-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**ATGGACGAAATGCTTCACCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3216	ARO8a-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GCACGACATTCTCATCATCG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3217	ARO8c-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GAACAGCTCAACAACTGGGT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3218	ARO8i-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GTCCGTCGGTATCAAAGTTG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3219	ARO9a-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**AGAAGCAGTACTCCTCAACA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3220	ARO9i-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GACAACATTAAATCTGTCGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3221	ARO9k-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GTGGACCTGCTCATTAACCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3306	AAT1-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**GCAGTGGCGAAAGATTGAGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3302	AAT2-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**AAGCAGATCTTCGAGAACGT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3006	URA3-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**CACGAGCTTGAGCACTCGAG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
3295	HMG2-20bp	TCGATGGGCCCCCGGTTCGATTCCGGGTCGGCGCA**CACTTCGATATTCCTGACCG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC

* – The variative region of recombineering oligonucleotides is underlined.

Table S17 Assembly of plasmid constructs (*continued on the next page*)

Module (Lvl)	Assembled plasmid	Backbone plasmid	Part 1	Part 2	Part 3	Manual section describing assembly (or GenBank file)
Exp (Lvl0)	pGenC-YlHPD1	pYTK001	OE-PCR from W29 by primers 3193, 3194, 3195, 3196			1.2
Exp (Lvl0)	pGenA-ScARO4^{K229L}	Gene synthesis				(GenBank file is provided)
Exp (Lvl0)	pGenK-ScARO7^{G141S}	Gene synthesis				(GenBank file is provided)
Exp (Lvl1)	pZUS1.1-HPD1	pZUS1.1	pProC-TEF1	pGenC-YlHPD1	pTerC-LIP2	1.3.3
Exp (Lvl1)	pZUS1.2-ARO4	pZUS1.2	pProC-TEF1	pGenA-ScARO4^{K229L}	pTerC-LIP2	1.3.3
Exp (Lvl1)	pZUS1.3-ARO7	pZUS1.3	pProC-TEF1	pGenK-ScARO7^{G141S}	pTerC-LIP2	1.3.3
Exp (Lvl1)	pB8US1.1-hrGFP	pB8US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pB11US1.1-hrGFP	pB11US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pC2US1.1-hrGFP	pC2US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pC7US1.1-hrGFP	pC7US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pC13US1.1-hrGFP	pC13US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pC14US1.1-hrGFP	pC14US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pD6US1.1-hrGFP	pD6US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pD12US1.1-hrGFP	pD12US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pE6US1.1-hrGFP	pE6US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pE8US1.1-hrGFP	pE8US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pE12US1.1-hrGFP	pE12US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3

Exp (Lvl1)	pE15US1.1-hrGFP	pE15US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pE16US1.1-hrGFP	pE16US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pF8US1.1-hrGFP	pF8US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pF9US1.1-hrGFP	pF9US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl1)	pF11US1.1-hrGFP	pF11US1.1	pProC-TEF1	pGenC-hrGFP	pTerC-LIP2	1.3.3
Exp (Lvl2)	pZUA2.3-HPD1-ARO4-ARO7	pZUA2.3	pZUS1.1-HPD1	pZUS1.1-ARO4	pZUS1.1-ARO7	1.3.4
Pro	pProUA-ALK1	pProUA-mScarlet	PCR from W29 by primers 3459 and 3460			1.8
Del	pDelUK-AAT1	pDelUK-RG	PCR from W29 by primers 3369 and 3370	PCR from W29 by primers 3371 and 3372		1.4
Int	pE8US-HPD1-ARO4-ARO7	pE8US1.1	pZUA2.3-HPD1-ARO4-ARO7			1.5.1
Int	pDelUK-AAT1::HPD1-ARO4-ARO7	pDelUK-AAT1	pE8US-HPD1-ARO4-ARO7			1.5.2
Int/MEx	pE8A1.1-HPD1	pE8UA2.2	pZUS1.1-HPD1			1.5.1/1.6
Int/MEx	pE8A1.2-ARO4	pE8UA2.2	pZUS1.2-ARO4			1.5.1/1.6
Int/MEx	pE8A1.3-ARO7	pE8UA2.2	pZUS1.3-ARO7			1.5.1/1.6
Int/MEx	pE8S-HPD1-ARO4-ARO7	pE8US1.1	pZUA2.3-HPD1-ARO4-ARO7			1.5.1/1.6
MEx	pC2S1.1-hrGFP	pC2US1.1-hrGFP				1.6
MEx	pD12S1.1-hrGFP	pD12US1.1-hrGFP				1.6
MEx	pE8S1.1-hrGFP	pE8US1.1-hrGFP				1.6
Cas	pCasNA-Rdm1	pCasNA-RK	Oligo 3149			1.7
Cas	pCasNA-Rdm2	pCasNA-RK	Oligo 3150			1.7
Cas	pCasNA-Rdm3	pCasNA-RK	Oligo 3151			1.7
Cas	pCasNA-ARO8a	pCasNA-RK	Oligo 3216			1.7
Cas	pCasNA-ARO8c	pCasNA-RK	Oligo 3217			1.7
Cas	pCasNA-ARO8i	pCasNA-RK	Oligo 3218			1.7
Cas	pCasNA-ARO9a	pCasNA-RK	Oligo 3219			1.7
Cas	pCasNA-ARO9i	pCasNA-RK	Oligo 3220			1.7
Cas	pCasNA-ARO9k	pCasNA-RK	Oligo 3221			1.7
Cas	pCasNA-AAT1	pCasNA-RK	Oligo 3306			1.7
Cas	pCasNA-AAT2	pCasNA-RK	Oligo 3302			1.7
Cas	pCasNA-URA3	pCasNA-RK	Oligo 3006			1.7
Cas	pCasNA-HMG2	pCasNA-RK	Oligo 3295			1.7

Structure of hybrid promoters

Sequence and structure of 43 hybrid promoters tested in the study are provided. Each promoter consists of several fragments which are parts of natural promoters derived from seven different yeast genomes. The source of each fragment (yeast species and gene name) is specified above the sequence panel. Corresponding DNA fragments and sources are highlighted using the same colours.

Hybrid promoter P1

S. cerevisiae PFK2 *O. polymorpha PYK1* *K. phaffii GND2*

AAAACGAAGATTAAGATAAAGTTGGGTAAAATCCGGGGTAAGAGGCAAGGGGGTAGAGAAAAAAAAA
CCGGAGTCATTATATACGATACCGTCCAGGGTAAGACAGTGATTTCTAGCTTCCACTTTTTTCAATTTCTT
TTTTTCGTTCCAAATGGCGTCCACCCGTACATCCGGAATCTGACGGCACAAGAGCCGATTAGTGGAAGC
CACGGTTACGTGATTGCGGTTTCGCGGCGACCGTCGCACTATGTCCCGCACGCGCCGCTTGGTAGACTG
GCCAGCGTCGTCCTGGCTGTGTGCATCGCTTTTGTTGAGCTCGTGTTGGAATTTCAGTATAAGCGATTCC
GTCAGACTCACTGCATCTGGGTCAGAGGGAGGCTTTAGGACAGGAGCCATCTGTACAGAGGCTACGGA
GTGTGGTGGCGGGTTCATGGGTGGCTCAAGCGGTCTAAAAGCAAAGGTGCGCGGCCGTACGTTATTGT
TTGTGTGGGTACGCGATAAATAAAAAATCAGACAATCGGCTATGGGGGTGACATAAGCGATGAGCAAA
CTCTATAGCCTCGGCAAACAGTCTCTTCCCTTGTCCCTCATTGATACCTCTTTATTCTCCCCCACCACCATA
CACTACCTTCCTCGCACCCCTGTCATCACAACCGCAATATAATTGATGCGCGGTTTCTTGCCTAATCCATC
GTCCAACAGAGAGGTCGCTCTCCTTATATATATAGTTGATCCCCCTTTTTTTCTACCCTTGCAATTTTTTTTT
GGGACCAAAGAAAAGAAACAAGACTGATACAAAA

Hybrid promoter P2

K. phaffii ACO1 *K. phaffii IDP2* *K. phaffii GND2*

GGTAAATACTTTTCTAGTGCACCCCGACATTCGACTCAAAAGGCTTAAACTAAACTCCTAAAATGTCCGT
GGTTGACCAATAGAAAGTATCACTCAGCTCCCTGATTGTTCATAGCCTAACTGTTTCTGAATCTCTCCAAG
TTTATTGCTGTCGGGTGAGCCTATGATTATCCCCTTTCACAATAGGCTCATTGTGTCTTAGGAAGTACCTG
CCCACTTCCCCCTGATAAACTTTCCACCATCCCCGGTCATTCGCTCATGACCTTGTTATTACCATGCCAAAA
CATCCCATAATGAAAGGGTATCGGCAACATGGGAGCTAAATTTCAGACCCTCGAGATGGAGTCGGTAAT
CGTTCGAGAACAATTTAACAGGTACAATGGTTATATGCAAGGAGCCGAGAAATTAACTTGGAGTTTTGG
TTCTGTTTGGAGTTGTCTCCGTTGGAGGAACAGAATACTAAACTTACTTGCAACAATTAATGAATAGGGT
GTAGGTTTTGGACATGAATACTATTGGTAGATGATAAAGAGTGAGGTCGTAGCTTGTAGTAACTGTAAT
CGTTAAGGGGTGTTCTTGATGATCTTGTATTGATACCTCTTTATTCTCCCCCACCACCATACACTACCTTCC
TCGCACCCCTGTCATCACAACCGCAATATAATTGATGCGCGGTTTCTTGCCTAATCCATCGTCCAACAGA
GAGGTCGCTCTCCTTATATATATAGTTGATCCCCCTTTTTTTCTACCCTTGCAATTTTTTTTTGGGACCAAA
GAAAAGAAACAAGACTGATACAAAA

Hybrid promoter P3

K. phaffii MDH1 *K. phaffii PFK1* *K. phaffii GND2*

TGAGGTGAAATATTCTTTAGTAATCTAGCAGGGAATCCTTGGAAGGGTAATTTAGACGGAAGACGTCAT
CTTGCGGAAGGGTGAAACATTCCAAGCAGAGTGCTGGTTCCGAGATTCTCCGCTCCTGTAGAAACCTTC
AACCTTCCAACATTAGAACCTTCGCGAATAGACTCCCTATCCAATCACGTTTCTCCGTTTCAAGGAAGTAG
CCTGGGTGTCTATGGTTAATATGTTCCTGCAGCGCGACTAATACTACGAATTTGCGCGCCTGCAACTCTC
CTCTCTCAGTCTGGTTCTTGAAGATGTGTATAAGTCTCGCAATGAAACAAATGTTCTGGGAGATGGATCG

TTGCTCAATATGAATATCGGATTAGTAAAGTATAAACTTATAAGACTTTCAGTGGTTTTCACATTGACCTA
GGCTTCTTATAACAAGGCGACTCCGAGAACAAAAGAAAAATAGAATGGCCCTCAAAGTAGCTTATGTGA
GATCTGGGCTTGATTCCAATCATAGTCCCTAGTTGATTGCTTGTAGCAAATGCCACAACAGTAGGCATTT
ACGTCCTCACAGTCTCTTCCCTTGTCCCTCATTGATACCTCTTTATTCTCCCCCACCACCATACACTACCTTC
CTCGCACCCCTGTCATCACAACCGCAATATAATTGATGCGCGGTTTCTTGCCTAATCCATCGTCCAACAGA
GAGGTCGCTCTCCTTATATATATAGTTGATCCCCCTTTTTTTCTACCCTTGCAATTTTTTTTTGGGACCAAA
GAAAAGAAACAAGACTGATACAAAA

Hybrid promoter P4
K. phaffii TPI1 *O. polymorpha ENO2* *K. phaffii GND2*

GAAATATACCACATTGCCAGTTTATACAGATGGTTAAGGGTGAAAATCAACGTTACACCTTGACGACCCC
ATTATTACGATGGCGTGAAGGAGATGAAGACCGGGTAGAAGAAATAAGAAAAGCGGTACAGTTTAGGT
CCGGAGATCTAGGGAAGGAGGCCTTAGCTTATATTGTAGCTGCTGAGAGAGAGGCAGCTGCTGGAAGA
TCTGAAGGCCCTATCAACCTCCTTTGAAAGGTCCCCTCTGGCAACAATAGTCAAATTTGTGCCGCGTTCT
GGAGGGTTAGGCGCAACATCCACATGAGTCAGTTTGAGGAGTTGAGAAGACGAGGAGTCGCATAGAA
GAAGGGGAGAGTCGCCCGGAACAGGCTTGGTGTCCTGGCCTGGAAAAGCAAACGGGTTGAGCGACAG
ACCGTTGACAAGAGAGAGCAAAAACACTAAAGGCACAGGGAAAAGCATTAGGAAGCAAGCACTGAAA
TTGTTGGCCTTAAAAGTCAATTGAGTTCCAATCATAGTCCCTAGTTGATTGCTTGTAGCAAATGCCACAA
CAGTAGGCATTTACGTCCTCACAGTCTCTTCCCTTGTCCCTCATTGATACCTCTTTATTCTCCCCCACCACC
ATACACTACCTTCCTCGCACCCCTGTCATCACAACCGCAATATAATTGATGCGCGGTTTCTTGCCTAATCC
ATCGTCCAACAGAGAGGTCGCTCTCCTTATATATATAGTTGATCCCCCTTTTTTTCTACCCTTGCAATTTTT
TTTTGGGACCAAAGAAAAGAAACAAGACTGATACAAAA

Hybrid promoter P5
O. polymorpha ACO1 *K. phaffii PGK1* *K. phaffii GND2*

GGTCACAATGTTTAAAGCTCTGTCGGTAAATTGTGATTGATCCATGATTAGCAAAATTGCAGTTGTAGGG
AAAGCAATTTATACAGCGTACAGACATCAGACGCTTGTGATGGTCTGGTGATCTAGAGCCGCACAGAAA
CTTCTAGCAATATCTGGTGTGTCTGGGTGGTTGCTGGTGTCTGGTCTGCATAACTTGGCCTGAGGATGCT
TATGTAATATGGCCTTCCTGGTGCATGGAAGTGGCTGGTGGCAAAAAAAAGTCTGTGAGCCTCCCAAGC
AATCTACTAATGTTTATTTTTCGTCCAACCTAATTGTGGTTTCAAAGCGCTATCAGGTGGGGGGTAAGAG
GAATGTGAGTGGAAAGCGAAAATAACTGGCAGCTGGGGTCAGATCCCGTGATGCCACCTCTTGTGGTA
TTTTGAAACGCGTGTTGCGATTGGCCGCGAGAACGGAAAGGAATATATTTACTGCCGATCGCATTTTGG
CCTCAAATAAATCTTGAGCTTTTGGACATAGATTATATGTTCTTTCTTGGAAGCTCTTTCAGCTAATAGTG
AAGTGTTTCCTACTAAGGATCGCCTCCAAACGTTCCAACTACGGGCGGAGGTTGCAAAGAAAACGGATC
TCTCAGCGAATTGTTCTCATCCATGAGTGAGTCCTCTCCGTCCTTTCCTCGCGCCTGCCTAATCCATCGTCC
AACAGAGAGGTCGCTCTCCTTATATATATAGTTGATCCCCCTTTTTTTCTACCCTTGCAATTTTTTTTTGGG
ACCAAAGAAAAGAAACAAGACTGATACAAAA

Hybrid promoter P8
O. polymorpha TPI1 *O. polymorpha PYK1* *K. phaffii ADH1*

TGTGTTGGTGCTGGTGACTCATGGAATATGGGCGCGTGTCTTCCTGACCAAATGGTTTAGATGGAGCGT
AGAAAAGTTCGAAGACCTGCAAAACGTGCCTAACTGTCGCTGGATAGTGATGGAAAAGGACGACACGA
CGCAGCGTTACGTCCTTCGCACGCATCTGAGCACGTGGTCAGAGCTTGAACAGACAAAAAGAGAGGAA
GAAGTCAAAAGAGATGCCGGGAGAGAGTTTACATTCAACAGTACAGTGCCTCTCACAGACGACGAGGT

AAGACAGGTAGCCGACGCGGAGGCGCGAGCGAAAAACGACCAGGTGCAAAAATCGCTAAAAATAAGC
GATTCCGTCAGACTCACTGCATCTGGGTCAGAGGGAGGCTTTAGGACAGGAGCCATCTGTACAGAGGC
TACGGAGTGTGGTGGCGGGTTCATGGGTGGCTCAAGCGGTCTAAAAGCAAAGGTGCGCGGCCGTACGT
TATTGTTTGTGTGGGTACGCGATAAATAAAAAATCAGACAATCGGCTATGGGGGTGACATAAGCGATGA
GCAAACTCTATAGCCTCGGCAAACCTGGGCGTGCACGGATGTCGTCGGAGTGCAATTTTCCAGCGGACC
AAAGTTCACCAGGAAAAAAATGACCCAATATGGGCGGTGCCCAATGATCACACCAACAATTGGTCCACC
CCTCCCCAATCTCTAATATTCACAATTCACCTCACTATAAATACCCCTGTCCTGCTCCCAAATTCTTTTTTCC
TTCTTCCATCAGCTACTAGCTTTTATCTTATTTACTTTACGAAA

Hybrid promoter P10
K. phaffii MDH1 *O. polymorpha ENO2* *O. polymorpha TAL1*

TGAGGTGAAATATTCTTTAGTAATCTAGCAGGGAACTCTTGGAAGGGTAATTTAGACGGAAGACGTCAT
CTTGCGGAAGGGTGAAACATTCCAAGCAGAGTGCTGGTTCCGAGATTCTCCGCTCCTGTAGAAACCTTC
AACCTTCCAACATTAGAACCTTCGCGAATAGACTCCCTATCCAATCACGTTTCTCCGTTTCAAGGAAGTAG
CCTGGGTGTCCGGTACGCCAAAAAACCTCCTTTGAAAGGTCCCCTCTGGCAACAATAGTCAAATTTGTGC
CGCGTTCTGGAGGGTTAGGCGCAACATCCACATGAGTCAGTTTGAGGAGTTGAGAAGACGAGGAGTCG
CATAGAAGAAGGGGAGAGTCGCCCGGAACAGGCTTGGTGTCCTGGCCTGGAAAAGCAAACGGGTTGA
GCGAGACACCGTTGACAAGAGAGAGCAAAAACACTAAAGGCACAGGGAAAAGCATTAGGAAGCAAGC
ACTGAAATTAGAAAGGAGGCAAATAATTTGTCACCCAGCTGGCTCCTAAAATGGAAATGCATGTGGCAA
AGTAATGGCTCCTAGGGTGGACTTCAAGGATACACTCACATTGATTGGTTGACAAGGTTACGTAAACTT
GTCTCCGAAGACGAATTTGTTTACGAAGATAGCATTTATTTGTAGGTTATTGATTAGGTAAGATTTATTC
AACGCAAACTGGGTGATGAATATAAATAAGCTCGGGTATTCCTCCGCAACCAGAACTTGAATTATATAC
GTTTCGATTGATTTCCATCAAATTATAAATTCAACAATTGCA

Hybrid promoter P11
S. cerevisiae ENO1 *K. phaffii GPM1* *Y. lipolytica GPDH*

AGTTAGTAGCAACCTGAACTCGGTCATTGATGCATGCATGTGCCGTGAAGCGGGACAACCAGAAAAGTC
GTCTATAAATGCCGGCACGTGCGATCATCGTGGCGGGGTTTTAAGAGTGCATATCACAAATTGTCGCAT
TACCGCGGAACCGCCAGATATTCATTACTTGACGCAAAAGCGTTTGAAATAATGACGAAAAAGAAGGAA
GAAAAAAAAAGAAAAATACCGCTTCTAGGCGGGTTATCTACTGATCCGAGCTTCCACTAGGATAGCACC
CAAACAGCTGCATATTTGGACGACCTTTACCAAATGAGAACCCTGTATTTCCTTGGCCAGAGCCTGAGAA
ACTGAACCCTGATCCAGACATTATCAATACCTTGGGTTATTAGTAGTGTCCGTTATTTTTCTGTTTAGGTT
ACGATTTTGCCAGATTTTTTGGGAGGAGGGAAACAAAAGAACCAGTGCTACACGACCTTTAAGTGCCAT
CAGGCATCCTGTTTTCTCGACCTCATCTCATCACATCCGTCAGTCTGAGCTTTCAGTTCTCAGTTTTCGATT
GACTCTTGCCCTGCTGCGCGCACACCATACCCTGGCTCCCTCTCATGCTTCTGGCGTTACCCCGGAATCGT
ACATCCATGCCGCGAATCCCGGACAGGACTCAGACGGATTTCACTATTTGGGCGGGCTTGCTCCGTCTG
TCCAAGGCAACATTTATATAAGGGTCTGCATCGCCGGCTCAATTGAATCTTTTTTCTTCTTCTCTTCTCTAT
ATTCATTCTTGAATTAAACACACATCAACA

Hybrid promoter P12
K. phaffii TPI1 *S. cerevisiae ADH1* *Y. lipolytica PFK1*

GAAATATACCACATTGCCAGTTTATACAGATGGTTAAGGGTGAAAATCAACGTTACACCTTGACGACCCC
ATTATTACGATGGCGTGAAGGAGATGAAGACCGGGTAGAAGAAATAAGAAAAGCGGTACAGTTTAGGT
CCGGAGATCTAGGGAAGGAGGCCTTAGCTTATATTGTAGCTGCTGAGAGAGAGGCAGCTGCTGGAAGA

TCTGAAGGCCCTATCACGTATGATGATGGTGATGACCATTAGAGAACGCCCAGAGATTGATAGCCAGTT
CTTGGACAACAATTCGGAACTTTATTCACGGTGCAAGTTTCACTACCCTTTTTCCATTTGCCATCTATTGA
AGTAATAATAGGCGCATGCAACTTCTTTTCTTTTTTTTTCTTTTCTCTCTCCCCCGTTGTTGTCTCACCATAT
CCGCAATGACAAAAAAATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGA
TGTCGTTGTTCCAGAGCTGATGAGGGGTATCTCGAAGCACACGAAACTTTTTCCTTCCTTCATTCACGCA
CACTACTCTCTAATGAGCAACGGTATACGGCCTTCCTTCCAGTTACTTGAATTTGAAATAAAAAAAAGTTT
GCTGTCTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGGCCGGCGTAACTTTGAGTGTCGT
CAAACTCTGATATATATATAGAGAGAGGTATCCCAACAGTTGATAGTCGACAAACGCAAAACAGACGGA
CACTGAACCCCCCGCGCTTCAAAACACCGACA

Hybrid promoter P13
Y. lipolytica PGK1 *K. marxianus MDH1* *K. phaffii AOX1*

CAGACAGTGACGAGTCATACATTCTCCGTATAATATCGTGTATGTCCAGACGATAGTCGTACTCGTACTC
GTTACTGTAACTACTGTGCGAGTACTCGTGCATGTATCGTAGGTATTGTATGTTCGAGTACATACACATA
CGATACCAAACACTGCCCACTGTTCTGTCATGTTAGATCATGGCCAATCCACGTGACTTGCATGCAGGTT
TGGCATTGAATATTCAGCGTGGCTACTACAAGTAGTACATACTGTATCAATACGATTGTACATACGGTAC
TCACCCTTTGCTACAGTATGTACATACAAGGGCGCACATGGACCTTGCCCTTTTGACTAGGGTACTACTC
TGGGCAGGGTACTTACTAAACTGGGCCCAGACAGACAGAGGCCCAGACAGAGGCCCCAAAGAGAAGC
CGCCAGGTTCCCTGCCTAGGCCTTCCTCTGGTTGCCCCCCAGAGTACCCTCCGGAACGAAACGAAACGA
AAACGCACATCGTGATGCGCGCTATTATAATTGCGTCTTGCGAATTCCATACGCCATGCGCTTAGTTAGC
ACATGAAGATTCTGGTGGGAATACTGCTGATAGCCTAACGTTCATGATCAAAATTTAACTGTTCTAACCC
CTACTTGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTATCATCATTATTAG
CTTACTTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAACGACAACTTGA
GAAGATCAAAAAACAACTAATTATTCGAAACA

Hybrid promoter P14
Y. lipolytica GPDH *O. polymorpha ENO2* *K. phaffii AOX1*

GTAGGTTGGGTTGGGTGGGAGCACCCCTCCACAGAGTAGAGTCAAACAGCAGCAGCAACATGATAGTT
GGGGGTGTGCGTGTTAAAGGAAAAAAAAAGAAGCTTGGGTTATATTCCCGCTCTATTTAGAGGTTGCG
GGATAGACGCCGACGGAGGGCAATGGCGCCATGGAACCTTGCGGATATGGGGACGCCGCGGCGGACT
GCGTCCGAACCAGCTCCAGCAGCGTTTTTTCCGGGCCATTGAGCCGACTGCGACCCCGCCAACGTGTCTT
GGCCCACGCACTCATGTCATGTTGGTGTTGGGAGGCCACTTTTTAAGTAGCACAAGGCACCTAGCTCGC
AGAAGGGGAGAGTCGCCCGGAACAGGCTTGGTGTCCTGGCCTGGAAAAGCAAACGGGTTGAGCGAGA
GAGCGTTGACAAGAGAGAGCAAAAACACTAAAGGCACAGGGAAAAGCATTAGGAAGCAAGCACTGAA
ATTAGAAAGGAGGCAAATAATTTTTGAAAGCGACAATGCGTCGACAGAATCTGCCGACAGAAAGATTG
TCGACAAGCGACGATAACGCAATAGTACATGGTGTTATTACGCAGTGTAACGTTCATGATCAAAATTTAA
CTGTTCTAACCCCTACTTGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTAT
CATCATTATTAGCTTACTTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAA
CGACAACTTGAGAAGATCAAAAAACAACTAATTATTCGAAACA

Hybrid promoter P23
Y. lipolytica TEF1 *Y. lipolytica ZWF1* *K. marxianus CYC1*

GGTATTTTCACAATTGCACCCCAGCCAGACCGATAGCCGGCCGCAATCCGCCACCCACAACCGTCTACCT
CCCACAAAAAGAAGTCACTTCCACCCTTTTCCACCAGATCATATGTCCCAACTTGCCAAATTAAAACCGTG

CGAATTTTCAAAATAAACTTTGGCAGGAAGGCTGCAAAGGAGGGGCTGGTGAGGGCGTCTGGAAGTCG
ACCACACACCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAATTGACCCCAAAT
TGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCACCCCACATATCAAACCTCCCCCGGTTCCC
ACACTTGCCGTTAAGGGCGTAGGGTACTGCAGTCTGGATAGTTCACCAGTCACGCTGGTGGGCCATTCT
CTTAGCACATTTCCCCCCTCCCAAGTCCCCTCAACCCCCAATGTAACCCTCAACCTCCCACAGCTCAGTAG
CACACGTGCAACTAGTTAGTAACAACCCCCCTCCGTCCAGCTTCTCTTTCACACTGCTTAGAGTTCGGGTG
CTACGAAATAGAACGCTCATAGTAGATGAAAAATTTCGTCAAAGCTAAAGCATTAATGCAGCATTTTGAT
ATATATAAGATGCTTCAAGAAGAAAAAAAAAGTCTTGAACTTTTATTCTTTACACTTGAGTGTGTTTTAAC
ATTCAAGCTAATCTGTTTAGTCAGTTTATAAACATCAAGCAACTGTTTGAATTTTATCATACGCAGCTAAA
CCAAACACACATTATTTATAGATAAGAA

Hybrid promoter P24
Y. lipolytica GPDH *Y. lipolytica ENO2* *K. marxianus TAL1*

GTAGGTTGGGTTGGGTGGGAGCACCCCTCCACAGAGTAGAGTCAAACAGCAGCAGCAACATGATAGTT
GGGGGTGTGCGTGTTAAAGGAAAAAAAAAGAAGCTTGGGTTATATTCCCGCTCTATTTAGAGGTTGCG
GGATAGACGCCGACGGAGGGCAATGGCGCCATGGAACCTTGCGGATATCCATACGCCGCGGCGGACTG
CGTCCGAACCAGCTCCAGCAGCGTTTTTTCCGGGCCATTGAGCCGACTGCGACCCCGCCAACGTGTCTTG
GCCCACGCACTCATGTCATGTTGGTGTTGGGAGGCCACTTTTTAAGTAGCACAAGGCACCTAGCTCGCA
GCAAGGTGTCCGAACCAAAGAAGCGGTCCCATCGGTAATCACGTGTGTGCCGATTTGCAAGACGAAAA
GCCACGAGAATAAACCGGGAGAGGGGATGGAAGTCCCCGAACAGCAACCAGCCCTTGCCCTCGTGGAC
ATAACCTTTCACTTGCCAGAACTCTAAGCGTCACCACGGTATACAAGCGCACGTAGAAGATTGTGGAAG
TCGTGTTGGAGACTGTTGATTTGGCTCTTGCCCATCCATTTTACTCATTATATAATCTAGCTTTTGAAATAT
AGGAAATAGGTATATAAAGGATAGGAAAAAAAATTGTAATTGCAGAAAAAGAGGTGAACACGGTTAAA
AACAGGTGAAAAACAGTTGTCAATTGTTGATGGATTGTTAGTAATTGATAGAATTTTCGATTAGAAATTG
TCTATTACCAATTGAGAATAAACACCACCAAACGATACAAGA

Hybrid promoter P25
S. cerevisiae PFK2 *S. cerevisiae ZWF1* *Y. lipolytica FBA1*

AAAACGAAGATTAAGATAAAGTTGGGTAAAATCCGGGGTAAGAGGCAAGGGGGTAGAGAAAAAAAAA
CCGGAGTCATTATATACGATACCGTCCAGGGTAAGACAGTGATTTCTAGCTTCCACTTTTTTCAATTTCTT
TTTTTCGTTCCAAATGGCGTCCACCCGTACATCCGGAATCTGACGGCACAAGAGCCGATTAGTGGAAGC
CACGGTTACGTGATTGCGGTTTTTTTTTCCTACGTATAACGCTATGACGGTAGTTGAATGTTAAAAACGA
AAACAGAGATATTGAATTGATCAATTTGATCAGTACGTATGTAATCTTTTGTTTGCCATGTTAAATCGGCC
TGAAATCACCACAAACTGTGTGTATCAAGTACATAGTGACATTTATATAATAGCAAGAACAACAATAATA
GTAGCGCTACTGGAAGCACCACGTAATAGTGGAAAAGAACTGGAAAAACCGCTATAAGATGCATACTC
CGGCGGTCTTACGCGGAGATACAAGCTTCCAACGGTGCTAAAAGCCCGGTAGTGTACTTCAATCGCCCC
CTGGATATAGCCCCGACAATAGGCCGTGGCCTCATTTTTTTGCCTTCCGCACATTTCCATTGCTCGGTACC
CACACCTTGCTTCTCCTGCACTTGCCAACCTTAATACTGGTTTACATTGACCAACATCTTACAAGCGGGGG
GCTTGTCTAGGGTATATATAAACAGTGGCTCTCCCAATCGGTTGCCAGTCTCTTTTTTCCTTTCTTTCCCCA
CAGATTCGAAATCTAAACTACACATCACACA

Hybrid promoter P26

Y. lipolytica GPDH *O. polymorpha PSH1* *O. polymorpha IDH2* *Y. lipolytica GPDH*

GTTGGGGTTACGTAATTGCGGCATTTGGGTCCTGCGCGCATGTCCCATTGGTCAGAATTAGTCCGGATA
GGAGACTTATCAGCCAATCACAGCGCCGAATCCACCTGTAGGTTGGGTTGGGTGGGAGCACCCCTCCAC
AGAGTAGAGTCAAACAGCAGCTCGCGACTCTCTGTCCGTTTCAGACTACACGAACATGCTTCTGGATGCC
GTCAAGTTCCATAACCATGAGTTTGGCATGGTAGTTTTGGACAGAATGGCAAAGGAATCGCCGCGCAGC
AACACCGAACTTCTCAGGGCTGTGTACGCCATAATCAAGTTTTACCTTGAGCTAGGGCGTCTGGACCTTG
CGATGCATATACTAGAGCAGGTCAAGAAGGAAAGTATGGGGATGTGGCAAACGATTCCATCGTTCTTAC
CAGCTGACAGGTCACTGGAAGAGCAATAGGTCGCAAAAGAACTGCCAAATCCCGCCGGACTTTGACTAC
ACTCCGATTAAGTTGTCGCGCGATACGTAGACTTTAGATGCCAATACGATGTGTGCTATGTTTATGTAAG
CGGGTCGATCGACCCCTGTCTCGTTGTATGCTCGCCAACGCCCGGTCTTTTGCACCACATCAGGTTACCC
CAAGCCAAACCTTTGTGTTAAAAAGCTTAACATATTATACCGAACGTAGGTTTGGGCGGGCTTGCTCCGT
CTGTCCAAGGCAACATTTATATAAGGGTCTGCATCGCCGGCTCAATTGAATCTTTTTTCTTCTTCTCTTCTC
TATATTCATTCTTGAATTAAACACACATCAACA

Hybrid promoter P28

Y. lipolytica TEF1 *Y. lipolytica ENO2* *Y. lipolytica PFK1*

GGTATTTTCACAATTGCACCCCAGCCAGACCGATAGCCGGCCGCAATCCGCCACCCACAACCGTCTACCT
CCCACAGAACCCCGTCACTTCCACCCTTTTCCACCAGATCATATGTCCCAACTTGCCAAATTAAAACCGTG
CGAATTTTCAAAATAAACTTTGGCAAAGAGGCTGCAAAGGAGGGGCTGGTGAGGGCGTCTGGAAGTCG
ACCACAGAGCGGGTTGGCGGCGTGGCACCCCGGAAAAAAACAGCCCCAATTGCCCCAATTGACCCCAA
ATTGACCCAGTAGCGGGCCCAACCCCGGCGAGAGCCCCCTTCACCCCACATATCAAATGGGCGAGAAG
GCGCGTAGATGTAGTCTTCCTCGGTCCCATCGGTAATCACGTGTGTGCCGATTTGCAAGACGAAAAGCC
ACGAGAATAAACCGGGAGAGGGGATGGAAGTCCCCGAACAGCAACCAGCCCTTGCCCTCGTGGACATA
ACCTTTCACTTGCCAGAACTCTAAGCGTCACCACGGTATACAAGCGCACGTAGAAGATTGTGGAAGTCG
TGTTGGAGACTGTTGATTTGGGCGGTGGAGGGGGGTATTTGAGAGCAATCCTTAACTGTTGGTCGGTTG
CCGTTTTTGCCTGCTCCTAACAGCTCGCACCGACTCTAAAAAACCTATACGACCTGGCCGGCGTAACTTT
GAGTGTCGTCAAACTCTGATATATATATAGACACACGTATCCCAACAGTTGATAGTCGACAAACGCAAA
ACAGACGGACACTGAACCCCCCGCGCTTCAAAACACCGACA

Hybrid promoter P32

S. cerevisiae ENO1 *S. cerevisiae ZWF1* *Y. lipolytica PFK1*

ATCGCGTTACCATATCGCCAAAAACTGATATACGCCGCGGAAACCAGGCAAACAATTGAAAAGAAAAAT
TTTGAGGAACTCTCTGCATCGAAGCCGTCTAGAGTTACCACTAGTCAGATGCCGCGGGCACTTGAGCAC
CTCATGCACAGCAATAACACAACACAATGGTTAGTAGCAACCTGAATTCGGTCATTGATGCATGCATGTG
CCGTGAAGCGGGACAACCAGAAAAGTCGTCTATAAATGCCGGCACGTGCGATCATCGTGGCGGGGTTT
TAAGAAAAGGATAACCCCTGTGTTGGGAGCACCTGGTAAGTAAGGTGTAGTTTTGCACCCGTGTACATA
AGCGTGAAATCACCACAAACTGTGTGTATCAAGTACATAGTGACATTTAATTAATAGCAAGAACAACAA
TAATAGTAGCGCTACTGGAAGCACCACGTAATAGTGGAAAAGAACTGGAAAAACCGCTATAAGATGCA
TACTCCGGCGGTCTTACGCGGAGATACAAGCTTCCAACGGTGCTAAAAGCCCGGTTTCGGCTCGGCCGG
AGGCGGTGGTTCAAACACCTCAGATCAGCTCACTATCGGCTGAGACAATCCTTAACTGTTGGTCGGTTG
CCGTTTTTGCCTGCTCCTAACAGCTCGCACCGACTATAAAAAACCTATACGACCTGGCCGGCGTAACTTT
GAGTGTCGTCAAACTCTGATATATATATAGACAGAGGTATCCCAACAGTTGATAGTCGACAAACGCAAA
ACAGACGGACACTGAACCCCCCGCGCTTCAAAACACCGACA

Hybrid promoter P34
Y. lipolytica STL1 *K. marxianus MDH1* *Y. lipolytica GND2*

TAAGTAGGCTTTTGAGTTTCCGTCTTTTTCGCAAAGTGAAAAAAGGTGGGATAATCCGGGGTGCCGTAC
ATTTGGAGAAGGTGGTAATATAACCGAATAGTTGGCCAATATGAGCCGCGAAAATCGGAATCAAGTCG
CCAACGTCTACTCATTTTATTCCAGATCTCAGATTCTTTCTAGCACATCTATAGGCCCTAATAACTGCGTCC
CCTTCGAGCTATAACATTTAAGGTGCAAAGAGAGGTTGGGAAGACAGCCTAAGGATTAGGGAGAGGTT
TTGCTGCGTGGGGGAACCGAGGGAGGGAATCCTCTGGAAGCCGGACCTTGCCCTTTTGACTAGGGTAC
TACTCTGGGCAGGGTACTTACTAAACTGGGCCCAGACAGACAGAGGCCCAGACAGAGGCCCCAAAGAG
AAGCCGCCAGGTTCCCTGCCTAGGCCTTCCTCTGGTTGCCCCCCAGAGTACCCTCCGGAACGAAACGAA
ACGAAAACGCACATCGTGATGCGCGCTATTATAATTGCGTCTTGCGAATTCCATACGCCATGCGCTTAGT
TAGCACATGAAATCCTGCCAATATTGACAATCCAACTTCCCAATTCCATTCCGATGACTAAAGTTTGAGC
GTTTAATCCGCCAAATGACCACACTGTATATAAACTCGATTGCCAGCGAAATAGAGTTGCTTTACTAAGC
ACAAAGTCTGTTGAGTTGGCTGAGACTTGGATTTATAAAAGGCTGCAGCGTCCCTCTCCAGACCTTTTCT
GCAACTTGACATTTTCTTGTTAACGACACCATCACACA

Hybrid promoter P36
S. cerevisiae ZWF1 *K. phaffii PGK1* *Y. lipolytica ADH1*

CCCGCCCGCTTTCGCTAGAGTACGTCTTCGCGCCCGTCAAGCAGGCCGTCGAAAAGGATCTAGCCCCTG
TTGGGAGCACCTGGTAAGTAAGGTGTAGTTTTGCACCCGTGTACATAAGCGTGAAATCACCACAAACTG
TGTGTATCAAGTACATAGTGACTTTTAAATAATAGCAAGAACAACAATAATAGTAGCGCTACTGGAAGC
ACCACGTAATAGTGGAAAAGAACTGGAAAAACCGCTATAAGATGCATACTCCGGCGGTCTTACGCGGA
GATACAAGCTATGTTTATTTTTCGTCCAACCTAATTGTGGTTTCAAAGCGCTATCAGGTGGGGGGTAAGA
GGAATGTGAGTGGAAAGCGAAAATAACTGGCAGCTGGGGTCAGATCCCGTGATGCCACCTCTTGTGGT
ATTTTGAAACGCGTGTTGCGATTGGCCGCGAGAACGGAAAGGAATATATTTACTGCCGATCGCATTTTG
GCCTCAAATAAATCTTGAGCTTTTGGACATAGATTATATGTTCTTTCTGCAATTGCACAATCGCAGACGGT
GGAGATTGTCGGGTGACGCACGTACACATACAGTATCGCCTGTACCACCCTACCCAGCACGTCAACTGC
AGCTGTTCAACAGACGTCGAACCCCCACCATCTCAAAGATGACAGGAGACAAGGAGACAGACTTTCTAT
CGTTTTTGAACCCTATTATTTCTGCCTCTTGCCTTATATAAAGGTCGGTCAGAGCGCCCTCTTATCATGGT
CCCCACTTCCAATCCCTACTTCTACACCAGCATCAAAA

Hybrid promoter P37
Y. lipolytica PGK1 *Y. lipolytica FBA1* *Y. lipolytica ENO2*

CAGACAGTGACGAGTCATACATTCTCCGTATAATATCGTGTATGTCCAGACGAAATGGTATGGATATGC
ACGGGGCGTAACTACTGTGCGAGTACTCGTGCATGTATCGTAGGTATTGTATGTTCGAGTACATACACA
TACGATACCAAACACTGCCCACTGTTCTGTCATGTTAGATCATGGCCAATCCACGTGACTTGCATGCAGG
TTTGGCATTGAATATTCAGCGTGGCTACTACAAGTAGTACATACTGTATCAATACGATTGTACATACGGT
ACTCACCCTTTGCTACATCACGTGTGTACAAGGGCGCACATGGCAGGCGCTGAGGTCGAGCAGGGTGG
TGTGACTTGTTATAGCCTTTAGAGCTGCGAAAGCGCGTATGGATTTGGCTCATCAGGCCAGATTGAGGG
TCTGTGGACACATGTCATGTTAGTGTACTTCAATCGCCCCCTGGATATAGCCCCGACAATAGGCCGTGGC
CTCATTTTTTTGCCTTCCGCACATTTCCATTTGAGATTTGTGCCATTGAGGGGGAGGTTATTGTGGCCATG
CAGTCGGATTTGCCGTCACGGGACCGCAACATGCTTTTCATTGCAGTCCTTCAACTATCCATCTCACCTCC
CCCAATGGCTTTTAACTATATAAAGACGAAAGCACCCCCCTTTGTACAGATGACTATTTGGGACCAATCC

AATAGCGCAATTGGGTTTGCATCATGTATAAAAGGAGCAATCCCCCACTAGTTATAAAGTCACAAGTATC
TCAGTATACCCGTCTAACCACACATTTATCACA

Hybrid promoter P38
O. polymorpha SDH1 *O. polymorpha PGI1* *Y. lipolytica ENO2*

CTCGCGACGGCGGACATTGGTGTCGCCATGGGCTCGGGGTCGGATCTGGCCTTGAGCTCGTGCGATTTT
GTCCTTTTGTCGCAGAAACAGCCACTCAAGCAGCTGCAAATCCTGCTCCAGCTGTCGCACAAAGTGATCA
ACCGTGTCAAATTCAACTTTGGCTGGGCACTGGTCTACAATGTGATTGGAATCCCGATCGCTGCAGGGG
TTATTTATCCGTACCATAACTCGCGTCTGAGTCCTACGTGGTCGAGTTTAGCCATGGCTTGTTCGAGCGT
GAGTGTTTTATGCAGCAGTCTGGCACTCAGATGGGATAATCAGCAGTCTTTTTCGGAATTTGAAAATGTT
TGGACGGACTACGAAAATAAGGAGTTTGATATTAATGAGAGTAAGTTTTTCAATGCAATGGAAGAGAA
GAACGAAGCTGCAGAAGAGCAAATAGACGCTTTGAAACAGCAGATCTTGAACATGGATGCATACACCA
AACAGCTCTGGGAGCAAGCAAAGTCGTCCTGGAAGTCCGGAAAATAGCCGCACAGCCCGGACGGTTTC
TTGCGGAGAACAGTTGCCGTCACGGGACCGCAACATGCTTTTCATTGCAGTCCTTCAACTATCCATCTCA
CCTCCCCCAATGGCTATAAAAGTTCGAATGACGAAAGCACCCCCCTTTGTACAGATGACTATTTGGGACC
AATCCAATAGCGCAATTGGGTTTGCATCATGTATAAAAGGAGCAATCCCCCACTAGTTATAAAGTCACAA
GTATCTCAGTATACCCGTCTAACCACACATTTATCACA

Hybrid promoter P43
Y. lipolytica PGK1 *K. marxianus TAL1* *K. lactis GPDH*

CAGACAGTGACGAGTCATACATTCTCCGTATAATATCGTGTATGTCCAGACGATAGTCGTACTCGTACTC
GTTACTGTAACTACTGTGCGAGTACTCGTGCATGTATCGTAGGTATTGTATGTTCGAGTACATACACATA
CGATACCAAACACTGCCCACTGTTCTGTCATGTTAGATCATGGCGGGGCCACGTGACTTGCATGCAGGTT
TGGCATTGAATATTCAGCGTGGCTACTACAAGTAGTACATACTGTATCAATACGATTGTATTTCCAATCCA
GATTCTACGGGACATAACAACAGCAACACCCACGATCCCACGCATATTTATTTCTTCTGTATCAGCTAGA
CTTTTTGTTCTCCTCACTGCTTGCTAGAGGCAGACAGACGTGTACAGCATTTGTAATCATTTATGACCTAC
TAGAAACAAAAAATAAAAAAAGGCATCAAAAAGCACACTACATTACATACACATGTATATACTACGAGC
CAAGCCAAGGCAAAGTAGGCCAGGGTAAGGTATGGTAAGGTAAGCGGCGGTATGCCCCACAGCCCTTA
GCAATTCCAGAAGTATCTGCACATAATGTGGTTATGTTCCAATATAGGTACCACCTTTGTTCAAGATTTA
GTTTTCTAATTGAATATAAATACAGAGGTTATTTCAACCTAATTGAGATTAAGGAGAGACTTATTTTACTA
TAGTATATATTTATATATAATTACTTATTGTTACTCCAATCCCCAAGTAGATTAGATTTAATCAATCACACA
CAAACATCAAAACAACAAATTAACAAAA

Hybrid promoter P45
Y. lipolytica ENO2 *Y. lipolytica GPDH* *Y. lipolytica ACO1* *Y. lipolytica ENO2*

TTCAGGCGGCTACTTGTATGTAGCATCCACGTTCATGTTTTGTGGATCAGATTAATGGTATGGATATGCA
CGGTTGAAATGAATCGGCCGACGCTCGGTAGTCGGAAAGAGCCGGGACCGGCCGGCGAGCATAAACC
GGACGCAGTAGGATGTCCTGCACGGGTCTTTTTGTGGGGTGTGGAGAGGGGGGTGCTTGGAGATGGA
AGCCGGTAGAACCGGGCTGCTTGGGGGGATTTGGGGCCGCTGGGCTCCAAAGAGGGGTAGGCATTTC
GTTGGGGTTACGTAATCCGCACCGAAGTGACAACATGGACAATGTGACACGTAGATACACGCAGGAAG
CAGCTGTCCACACACATTTATCCCGAAAAATAGCCCGCATCACATGCACGACTCGTAAAAAGAAAAGAG
CTGCGGGCCAAAGGACCAATAAGTGCCGAGGAATGTTAAGCCAAAAGAACAACGACGATCGCCAGACA
GGTTTAGTGGGAGCAGCAGCAGCAGAGGCCGTGCAACGGCAGGAGAGAGAGGTCTGGCGAAAAGGA
GGACAGGGGCCATGCAGTCGGATTTGCCGTCACGGGACCGCAACATGCTTTTCATTGCAGTCCTTCAAC

TATCCATCTCACCTCCCCCAATGGCTTTTAACTTTCGAATGACGAAAGCACCCCCCTTTGTACAGATGACT
ATTTGGGACCAATCCAATAGCGCAATTGGGTTTGCATCATGTATAAAAGGAGCAATCCCCCACTAGTTAT
AAAGTCACAAGTATCTCAGTATACCCGTCTAACCACACATTTATCACA

Hybrid promoter P46
K. phaffii CYC1 *Y. lipolytica GPDH* *Y. lipolytica ZWF1* *K. phaffii CYC1*

TCAATAAGGTTTTTGACAGTCTAAAACTTTCTATAACGAGTATGCCTTACATCTTGAAACGATGGCTTGG
ACCAGACCGAGGAGTAGCCGACGCTCGGTAGTCGGAAAGAGCCGGGACCGGCCGGCGAGCATAAACC
GGACGCAGTAGGATGTCCTGCACGGGTCTTTTTGTGGGGTGTGGAGAGGGGGGTGCTTGGAGATGGA
AGCCGGTAGAACCGGGCTGCTTGGGGGGATTTGGGGCCGCTGGGCTCCAAAGAGGGGTAGGCATTTC
GTTGGGGTTACGTAATTGCGGCATTTGGGTCCTGCGCGCATGTCCCATTGGTCAGAATTAGTCCGGATA
GGAGACTTATCAGCCAATCACAGCCTGCGTACCACCTGTAGGTGCGATAGTTCACCAGTCACGCTGGTG
GGCCATTCTCTTAGCACATTTCCCCCCTCCCAAGTCCCCTCAACCCCCAATGTAACCCTCAACCTCCCACA
GCTCAGTAGCACACGTGCAACTAGTTAGTAACAACCCCCCTCCGTCCAGCTTCTCTTTCACACTGCTTAGA
GTTCGGGTGCCAACGTTGCCAAACTACCCTGACGTGCTAACAAAGGAAAGCGTTTTAACTGTAAGCTAT
CAGATTACATAAGACCTGTCCGCGATCGCCCGATCTCAGAGTTCCCACAAGAGCCAATAAAATCGCGCG
AATTTGAAATCGGAAAATGTAGGAACTCAATTGTCCTATATAAATAATTCCCTTCCCGTGGAAACTGAAA
AAAAAGTTCACCCCGACTTATTTATTCAATTCAACACAAAA

Hybrid promoter P47
S. cerevisiae PGK1 *Y. lipolytica GPDH* *S. cerevisiae PGK1* *K. phaffii ADH1*

GCAAGAATTACTCGTGAGTAAGGAAAGAGTGAGGAACTATCGCATACCTGCATTTAAAGATGCCGATTT
GGGCGCGAAATGAATCGGCCGACGCTCGGTAGTCGGAAAGAGCCGGGACCGGCCGGCGAGCATAAAC
CGGACGCAGTAGGATGTCCTGCACGGGTCTTTTTGTGGGGTGTGGAGAGGGGGGTGCTTGGAGATGG
AAGCCGGTAGAACCGGGCTGCTTGGGGGGATTTGGGGCCGCTGGGCTCCAAAGAGGGGTAGGCATTT
CGTTGGGGTTACGTAATTGCGGCATTTGGGTCCTGCGCGCATGTCCCATTGGTCAGAATTAGTCCGGAT
AGGAGACTTATCAGCCAATGGCGGGAAAGGGTTTAGTACCACATGCTATGATGCCCACTGTGATCTCCA
GAGCAAAGTTCGTTCGATCGTACTGTTACTCTCTCTCTTTCAAACAGAATTGTCCGAATCGTGTGACAAC
AACAGCCTGTTCTCACACACTCTTTTCTTCTAACCAAGGGGGTGGTTTAGTTTAGTAGAACCTCGTGAAA
CTTACATTTACATATATATAAACTTGCATAAATTGGTCAATGCAAGAAATACATATTTTGCCTATTGTAGA
CGTCAACCCGCATCTGGTGCGAATATAGCGCACCCCCAATGATCACACCAACAATTGGTCCACCCCTCCC
CAATCTCTAATATTCACAATTCACCTCACTATAAATACCCCTGTCCTGCTCCCAAATTCTTTTTTCCTTCTTC
CATCAGCTACTAGCTTTTATCTTATTTACTTTACGAAA

Hybrid promoter P50
Y. lipolytica MDH1 *K. lactis PFK2* *K. phaffii AOX1*

GTTCCGGCTCCGATTTCTCGGCCAAGTGCCGACTCCACAAGACCGAGGAAGGTAAACTCTCTGTTCACGT
GGGGCTGAAGAAAGGCCAGCTCACGGTGGTGGAGTTGCAATGGAGGCGGCAGCTGAATAGACCTTGC
AAGTAAGGGGTACCTGACGGGAATGTGGGAATATGGGACACAAATTGGGGGGCAAACTTGCAACATG
CGTCCAGTGTACCCCGGATAACACCATTAGTGTGGCTAATAGCGAGGATGGGGACTTGGAGGGATGGG
TTCGCAGGGATGGACTCGGAGGGATGGACGCGAATGGCGTGGAGGGCTCGGATGGCGCGGAGGGTTC
GGAGATGGGTCCAGGGCCAAAAATCCGGTTTAAAAAGGTGGATATGGTCGATTGTAGTGGAAGCTCGT
GATCCTACATGCTCACGAAACCCATCATCATGCAATCCACATTAAAGGAAGGGAAAAGGATATTGAACT
TTTGACTATTTAGTATAAATGAAAACTACTTTGTAAGCTTGGACAGAGGAATAATTTCTGATTCGTGCTTC

TGCTTCTACTGACTTGCAAACCAGTAAACAAGTTACTGCATTAATCAGTAATAATCTGCGTATAGCGAAA
CTTGTAACCCTACTTGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTATCAT
CATTATTAGCTTACTTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAACGA
CAACTTGAGAAGATCAAAAAACAACTAATTATTCGAAACA

Hybrid promoter P55
Y. lipolytica ENO2 *O. polymorpha PYK1* *S. cerevisiae PGK1*

ATCACGCTACACTTAGCTACAGAATAAAGCTCGGTAGCGCCAACAGCGTTGACAAATAGCTCAAGGGCG
TGGAGCACAGGGTTTAGGAGGTTTTAATGGGCGAGAAGGCGCGTAGATGTAGTCTTCCTCGGTCCCATC
GGTAATCACGTGTGTGCCGATTTGCAAGACGAAAAGCCACGAGAATGGGGCGGGAGAGGGGATGGAA
GTCCCCGAACAGCAACCAGCCCTTGCCCTCGTGGACATAACCTTTCACTTGCCAGAACTCTAAGCGTCAC
CACGGTATACAAGCGCACGTAGAAGATTGTGGAAGTTGAGCTCGTGTTGGAATTTCAGTATAAGCGATT
CCGTCAGACTCACTGCATCTGGGTCAGAGGGAGGCTTTAGGACAGGAGCCATCTGTACAGAGGCTACG
GAGTGTGGTGGCGGGTTCATGGGTGGCTCAAGCGGTCTAAAAGCAAAGGTGCGCGGCCGTACGTTATT
GTTTGTGTGGGTACGCGATAAATAAAAAATCAGACAATCGGCTATGGGGGTGACATAAGCGATGAGCA
AACTCTATAGCCTCGGCAAACCTGGGCGTGCACACACTCTTTTCTTCTAACCAAGGGGGTGGTTTAGTTT
AGTAGAACCTCGTGAAACTTACATTTACATATATATAAACTTGCATAAATTGGTCAATGCAAGAAATACA
TATTTGGTCTTTTCTAATTCGTAGTTTTTCAAGTTCTTAGATGCTTTCTTTTTCTCTTTTTTACAGATCATCA
AGGAAGTAATTATCTACTTTTTACAACAAATATAAAACA

Hybrid promoter P62
S. cerevisiae TEF1 *K. lactis TEF1* *Y. lipolytica TEF1*

AGGTCTTTAGTCAGAGGCAGGAACAGCCGTCAAGGGGGCATAAGACTACGGTCATCCCCATCTGCCTCT
TCGTCCAGCCTTGCCAACAGGGAGTTCTTCAGAGACATGGAGGCTCAAAACGAAATTATTGACAGCCTA
GACATCAATAGTCATACAACAGAAAGCGACCACCCAACTTTGGCTGGGGGTAGCGTATAAACAATGCAT
ACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATTACATATAATACATATCACATAGGA
ATGGACAGACAACTATACCAGCATGGATCTCTTGTATCGGTTCTTTTCTCCCGCTCTCTCGCAATAACAAT
GAACACTGGGTCAATCATAGCCTACACAGGTGAACAGAGTAGCGTTTATACAGGGTTTATACGGTGATT
CCTACGGCAAAAATTTTTCATTTCTAAAAAGAAAAAGAAAAATTTTTCTTTCCAACGCTAGAAGGAAAAG
AAAAATCTAATTAAATTGATTTGGTGATTTTCTGAGAGTTCCCACACTTGCCGTTAAGGGCGTAGGGTAC
TGCAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACCCATGC
CGGACGCTATATAAGCTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAAAAGAC
CACCGTCCCCGAATTACCTTTCCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTTAAGCA
TTTCCTTCTGAGTATAAGAATCATTCAAA

Hybrid promoter P67
Y. lipolytica TEF1 *Y. lipolytica TEF1* *C. hispaniensis TEF1* *Y. lipolytica TEF1*

TTCGCTCCCACACTACACCGTAATACCACGTCACTCTCATTGCAGGTTACCCTGCCCGTAGTCGCTCGATC
CACCTCCTCCTTCTCTCGTGTGTGCAGCAAAGAGGCAGAGATGGAGCCCGTATGGTGAATTAAAACCGT
GCGAATTTTCAAAATAAACTTTGGCAAAGAGGCTGCAAAGGAGGGGGGGGTGAGGGCGTCTGGAAGT
CGACCAGACAGCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAAGTCCACCAC
CACTGGACACTTGATTTACAAGTGCGGTGGTATGGATAAGCGAACCATTGAAAAGTTTGAGAAGGAAG
CCGATGAGCTTGGAAAGGGTTCTTTCAAGTACGCTTGGGTTCTTGACAAGTTGAAGGCTGAGCGAGAGC
GAGGTATCACCATTGATATTGCTCTCTGGAAGTTCGACACGCCCAAGTACTACGTTACCATTATTGATGC

TCCCGGTCACCGAGATTTCATCAAGAATATGATTACCGGTACTTCCCCACACTTGCCGTTAAGGGCGTAG
GGTACTGCAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACC
CATGCCGGACGCTATATAAGCTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAA
AAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTT
AAGCATTTCCTTCTGAGTATAAGAATCATTCAAA

Hybrid promoter P68
S. cerevisiae TEF1 *C. hispaniensis TEF1* *Y. lipolytica TEF1*

AGGTCTTTAGTCAGAGGCAGGAACAGCCGTCAAGGGGGCATAAGACTACGGTCATCCCCATCTGCCTCT
TCGTCCAGCCTTGCCAACAGGGAGTTCTTCAGAGACATGGAGGCTCAAAACGAAATTATTGACAGCCTA
GACATCAATAGTCATACAACAGAAAGCGACCACCCAACTTTGGCTGGGGGTAGCGTATAAACAATGCAT
ACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATTACATATAATACATATCACATAGGA
AGCAACACTTGATTTACAAGTGCGGTGGTATGGATAAGCGAACCATTGAAAAGTTTGAGAAGGAAGCC
GATGAGCTTGGAAAGGGTTCTTTCAAGTACGCTTGGGTTCTTGACAAGTTGAAGGCTGAGCGAGAGCG
AGGTATCACCATTGATATTGCTCTCTGGAAGTTCGAGAGGCCCAAGTACTACGTTACCATTATTGATGCT
CCCGGTCACCGAGATTTCATCAAGAATATGATTACCGGTACTTCCCCACACTTGCCGTTAAGGGCGTAGG
GTACTGCAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACCC
ATGCCGGACGCTATATAAGCTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAAA
AGACCACCGTCCCCGAATTACCTTTCCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTTA
AGCATTTCCTTCTGAGTATAAGAATCATTCAAA

Hybrid promoter P73
S. cerevisiae TEF1 *K. lactis TEF1* *K. phaffii AOX1*

AGGTCTTTAGTCAGAGGCAGGAACAGCCGTCAAGGGGGCATAAGACTACGGTCATCCCCATCTGCCTCT
TCGTCCAGCCTTGCCAACAGGGAGTTCTTCAGAGACATGGAGGCTCAAAACGAAATTATTGACAGCCTA
GACATCAATAGTCATACAACAGAAAGCGACCACCCAACTTTGGCTGGGGGTAGCGTATAAACAATGCAT
ACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATTACATATAATACATATCACATAGGA
AGCAACAGACAACTATACCAGCATGGATCTCTTGTATCGGTTCTTTTCTCCCGCTCTCTCGCAATAACAAT
GAACACTGGGTCAATCATAGCCTACACAGGTGAACAGAGTAGCGTTTATACAGGGTTTATACGGTGATT
CCTACGGCAAAAATTTTTCATTTCTAAAAGAAAAAAGAAAAATTTTTCTTTCCAACGCTAGAAGGAAAAG
AAAAATCTAATTAAATTGATTTGGTGATTTTCTGAGAGTTCCCTTTTTCATATATCGAATTTTGAATATAA
AAGGAGATCGAAAAAATTTTTCTATTCAATCTGTTTTCTGGTTTTATTTGATAGTTTTTTCTAACCCCTACT
TGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTATCATCATTATTAGCTTAC
TTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAACGACAACTTGAGAAGA
TCAAAAAACAACTAATTATTCGAAACA

Hybrid promoter P78
S. cerevisiae TEF1 *K. phaffii TEF1* *S. cerevisiae HXT7*

TGAGGTCTTTAGTCAGAGGCAGGAACAGCCGTCAAGGGGGCATAAGACTACGGTCATCCCCATCTGCCT
CTTCGTCCAGCCTTGCCAACAGGGAGTTCTTCAGAGACATGGAGGCTCAAAACGAAATTATTGACAGCC
TAGACATCAATAGTCATACAACAGAAAGCGACCGCCCAACTTTGGCGGGGAATAGCGTATAAACAATGC
ATACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATTACATATAATACATATCACATAG
GAAGCAACAGGCGCGTTGGCCTCCTTCACCTTTTAACCATCTTGCCCATTCCAACTCGTGTCAGATTGCGT
ATCAAGTGAAAAAGAAAAAATTTTAAATCTTTAACCCAATCAGGTAATAACTGTCGCCTCTTTTATCTGCC

GCACTGCATGAGGTGTCCCCTTAGTGGAAAAGAATACTGAGCCAACCCTGGAGGACAGCAAGGGAAAA
ATACCTACAACTTGCTTCATAATGGTCGTAAAAACAATCCTTGTCGGATATAAGTGTTGTAGACTGTCCCT
TATCCTCTGCGATGTTCTTCCTCTCAAAGTTTGCGATTTCTCTCTATCAGAATTGCCATCAAGAGAAAAAA
AAGTATAAATAGACACCATATATGCCAATACTTCACAATGTTCGAATCTATTCTTCATTTGCAGCTATTGT
AAAATAATAAAACATCAAGAACAAACAAGCTCAACTTGTCTTTTCTAAGAACAAAGAATAAACACAAAA
ACAAAAAGTTTTTTTAATTTTAATCAAAAA

Hybrid promoter P88
Y. lipolytica TEF1 *K. marxianus TEF1* *Y. lipolytica TEF1*

GGTATTTTCACAATTGCACCCCAGCCAGACCGATAGCCGGCCGCAATCCGCCACCCACAACCGTCTACCT
CCCACAGAACCCCGTCACTTCCACCCTTTTCCACCAGATCATATGTCCCAACTTGCCAAATTAAAACCGTG
CGAATTTTCAAAATAAACTTTGGCAAAGAGGCTGCAAAGGAGGGGGGGGTGAGGGCGTCTGGAAGTC
GACCACACAGCGGGTTGGCGGCGTATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAATTGACCCCAA
ATTGACCCAGTAGCGGGCCAGCGTATCCCAGCCTAGTGTATCCCAGCCTAGCCTAGCCTAGGCCAAACC
TAGCCCTCTCTAGCCTAGCGCCCAGCAGAAACACCGATGAAGCAAAGAAGTAACAGCAGGAAAGAAAA
ACAAACACAACAAAAAAAAACAAGCAGCATAGCATCAACAGAAATTTCTAAAGAGAACCAAATTCACCC
CAGAAACAACCGCACAAATACGACATCCATCCACCTTTCTTTTATCCCACACTTGCCGTTAAGGGCGTAG
GGTACTGCAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACC
CATGCCGGACGCTATATAAGCTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAA
AAGACCACCGTCCCCGAATTACCTTTCCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTT
AAGCATTTCCTTCTGAGTATAAGAATCATTCAAA

Hybrid promoter P89
S. cerevisiae TEF1 *K. marxianus TEF1* *Y. lipolytica TEF1*

AGGTCTTTAGTCAGAGGCAGGAACAGCCGTCAAGGGGGCATAAGACTACGGTCATCCCCATCTGCCTCT
TCGTCCAGCCTTGCCAACAGGGAGTTCTTCAGAGACATGGAGGCTCAAAACGAAATTATTGACAGCCTA
GACATCAATAGTCATACAACAGAAAGCGACCACCCAACTTTGGCTGGGGGTAGCGTATAAACAATGCAT
ACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATTACATATAATACATATCACATAGGA
AGCAACAGGCGCCATCCCAGCGTATCCCAGCCTAGTGTATCCCAGCCTAGCCTAGCCTAGGCCAAACCT
AGCCCTCTCTAGCCTAGCGCCCAGCAGAAACACCGATGAAGCAAAGAAGTAACAGCAGGAAAGAAAAA
CAAACACAACAAAAAAAAACAAGCAGCATAGCATCAACAGAAATTTCTAAAGAGAACCAAATTCACCCC
AGAAACAACCGCACAAATACGACATCCATCCACCTTTCTTTTATCCCACACTTGCCGTTAAGGGCGTAGG
GTACTGCAGTCTGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACCC
ATGCCGGACGCTATATAAGCTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAAA
AGACCACCGTCCCCGAATTACCTTTCCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCGTTA
AGCATTTCCTTCTGAGTATAAGAATCATTCAAA

Hybrid promoter P92
O. polymorpha ZWF1 *Y. lipolytica PFK1* *O. polymorpha TAL1*

GTTGAGCACGACGCGACCCCGATCTCGCACATCTATGAGCAGTATGCGCCCCGGGACTGGCCCGATGCG
GAGGCGGCATGGCTCAACTGCGAAGGCTCGTCGTCAGGAGGGTATGCGCTCCAGTATTTATTGCACTTG
CAAAAAAAGCACGAAAACACAGAGATCAGGACGATAAACGGCAGAAGGGGGGACATTTACGAGCTGC
GGCTTGTGAGCGGGTCCGAGGTGTCTACTTGGTCCTGGTACTACACTCGTACCGGTACCTTTTGATCCGA
TGGTTACTTTTTATGTTCTATTTTACATTAGCGTGGAAATAGACCATGCCATCTTTGGCACCCCGGAAAAA

CTTGATCCAATAGAGTTGTTGGGTGGAGCTAGTGACTGGCGGCAATTGGAGAGCTTCTAGAAGACGAA
CCAGGAGCCCGATAGGAACTCCGTTGGCGTGAGTCGGCCCCCCAGTAGCAATTCGAATCACGTGACGTG
GAGTTTTCCCTCGCCCGCGTTCCTGGATTGTCCCAGCTGGCTCCTAAAATGGAAATGCATGTGGCAAAGT
AATGGCTCCTAGGGTGGACTTCAAGGATACACTCACATTGATTGGTTGACAAGGTTACGTAAACTTGTCT
CCGAAGACGAATTTGTTTACGAAGATAGCATTTATTTGTAGGTTATTGATTAGGTAAGATTTATTCAACG
CAAACTGGGTGATGAATATAAATAAGCTCGGGTATTCCTCCGCAACCAGAACTTGAATTATATACGTTTC
GATTGATTTCCATCAAATTATAAATTCAACAATTGCA

Hybrid promoter P94
Y. lipolytica GPDH *O. polymorpha PGI1* *O. polymorpha TAL1*

GTAGGTTGGGTTGGGTGGGAGCACCCCTCCACAGAGTAGAGTCAAACAGCAGCAGCAACATGATAGTT
GGGGGTGTGCGTGTTAAAGGAAAAAAAAAGAAGCTTGGGTTATATTCCCGCTCTATTTAGAGGTTGCG
GGATAGACGCCGACGGAGGGCAATGGCGCCATGGAACCTTGCGGATATGGGGACGCCGCGGCGGACT
GCGTCCGAACCAGCTCCAGCAGCGTTTTTTCCGGGCCATTGAGCCGACTGCGACCCCGCCAACGTGTCTT
GGCCCACGCACTCATGTCATGTTGGTGCGGCTTGCACGGGATAATCAGCAGTCTTTTTCGGAATTTGAAA
ATGTTTGGACGGACTACGAAAATAAGGAGTTTGATATTAATGAGAGTAAGTTTTTCAATGCAATGGAAG
AGAAGAACGAAGCTGCAGAAGAGCAAATAGACGCTTTGAAACAGCAGATCTTGAACATGGATGCATAC
ACCAAACAGCTCTGGGAGCAAGCAAAGTCGTCCTGGAAGCTGGCTCCTAAAATGGAAATGCATGTGGC
AAAGTAATGGCTCCTAGGGTGGACTTCAAGGATACACTCACATTGATTGGTTGACAAGGTTACGTAAAC
TTGTCTCCGAAGACGAATTTGTTTACGAAGATAGCATTTATTTGTAGGTTATTGATTAGGTAAGATTTATT
CAACGCAAACTGGGTGATGAATATAAATAAGCTCGGGTATTCCTCCGCAACCAGAACTTGAATTATATAC
GTTTCGATTGATTTCCATCAAATTATAAATTCAACAATTGCA

Hybrid promoter P95
S. cerevisiae TEF1 *Y. lipolytica ZWF1* *K. phaffii PYK1*

TATTTTCACAATTGCACCCCAGCCAGACCGATAGCCGGCCGCAATCCGCCACCCACAACCGTCTACCTCC
CACAGAACCCCGTCACTTCCACCCTTTTCCACCAGATCATATGTCCCAACTTGCCAAATTAAAACCGTGCG
AATTTTCAAAATAAACTTTGGCAAAGAGGATGATAAGGAGGGGGGGGTGAGGGCGTCTGGAAGTCGA
CCACACACGGGGTTGGCGGCGTATTTGTGTCCCAAAAAGCAGCCCCAATTGCCCCAATTGACCCCAAAG
CCCTACGTCTGGACCGCTACATTATTTCATAGCACAGCAACAGAACCGAGTTTAATGGTTTGAAACCTAG
GTGGAAGAGGGGCGGGCGAGGTATCGTACTGTGGGTGCGATAGTTCACCAGTCACGCTGGTGGGCCA
TTCTCTTAGCACATTTCCCCCCTCCCAAGTCCCCTCAACCCCCAATGTAACCCTCAACCTCCCACAGCTCAG
TAGCACACGTGCAACTAGTTAGTAACAACCCCCCACCGTCCAGCTTCCGTACGTTTTTCACATTCAAGGAT
GAGGGTTTTCCACGAGTGAACTATTACTCCGGACCCCCACCATCATTTGCGGAATGAAACCTTTTGTGCT
GAGATTATATAAGGCGTGGGGACGGACGCTTCTTAACCGTTCCCCTAGAATGTCGTCCCCTGATCAAAA
TTTAATGGCATCCAACTTTGCTGTAATAGGTATATATAACCTAGCAGGCGACCGTTCATGTACAGTAAAT
TGTTTTAGACTTTTTTTTAACTGAAATCAATCCA

Hybrid promoter P96
K. phaffii TPI1 *Y. lipolytica TPI1* *Y. lipolytica GND2*

CGAAATATACCACATTGCCAGTTTATACAGATGGTTAAGGGTGAAAATCAACGTTACACCTTGACGACCC
CATTATTACGATGGCGTGAAGGAGATGAAGACCGGGTAGAAGAAATAAGAAAAGCGGTACAGTTTAG
GTCCGGAGATCTAGGGAAGGAGGCCTTAGCTTATATTGTAGCTGCTGGGGGAGAGGCAGCTGCTGGAA
GATCTGAAGGCCCTATCACGTATGATGATGGTGATGACCATTAGAGAACGCCCAGAGATTGATAGCCAG

GTGACTCAGAAAGTGCATGTTGGAAATGAGCCACAGACCAAGACAAGATATGACAAAATTGCACTATTC
GATGCAGAATTCGACGGTGTTTCCATTGGTGTTATGACATTCATCTGCATTCATACAAAAAAGTCTTGGT
AGTGGTACTTTTGCGTTATTACCTCCGATATCTACGCACCCCCCAACCCCCCTGCTACAGTAAAGAGTGT
GAGTCTACTGTACATGCTTACTAACTCTCTCTCCAAGACGGCACATCTGAGCACCAGCCACAACCAACAA
ACGACTTCTACTTGAGTGTGGGGAGATTGCTCACAAGTCTGGACTGCCACGGATGACTAAAGTTTGAGC
GTTTAATCCGCCAAATGACCACACTGTGCATAAACTCGATTGCCAGCGAAATAGAGTTGCTTTACTAAGC
ACAAAGTCTGTTGAGTTGGCTGAGACTTGGATATATAAAACGCTGCAGCGTCCCTCTCCAGACCTTTTCT
GCAACTTGACATTTTCTTGTTAACGACACCATCACACA

Hybrid promoter P97
K. phaffii PMA1 *K. phaffii PGK1* *Y. lipolytica FBA1*

AGGAAACCTCGATGATTCTCCCGTTCTTCCATGGGCGGGTATCGCAAAATGAGGAATTTTTCAAATTTCT
CTATTGTCAAGACTGTTTATTATCTAAGAAATAGCCCAATCCGAAGCTCAGTTTTGAAAAAATCACTTCCG
CGTTTCTTTTTTACAGCCCGATGAATATCCAAATTTGGAATATGGGGTACTCTATCGGGACTGCAGATAA
TATGACAACAACGCAGATTACATTTTAGGTAAGGCATAAATTCTACAGGCACGTGCGAGGCAAGCAATC
TACTAATGTTTATTTTTCGTCCAACCTAATTGTGGTTTCAAAGCGCTATCAGGTGGGGGGTAAGAGGAAT
GTGAGTGGAAAGCGAAAATAACTGGCAGCTGGGGTCAGATCCCGTGATGCCACCTCTTGTGGTATTTTG
AAACGCGTGTTGCGATTGGCCGCGAGAACGGAAAGGAATATATTTACTGCCGATCGCATTTTGGCCTCA
AATAAATCTTGAGCTTTTGGACATAGGTCTGTGGACACATGTCATGTTAGTGTACTTCAATCGCCCCCTG
GATATAGCCCCGACAATAGGCCGTGGCCTCATTTTTTTGCCTTCCGCACATTTCCATTGCTCGGTACCCAC
ACCTTGCTTCTCCTGCACTTGCCAACCTTAATACTGGTTTACATTGACCAACATCTTACAAGCGGGGGGCT
TGTCTAGGGTATATATAAACAGTGGCTCTCCCAATCGGTTGCCAGTCTCTTTTTTCCTTTCTTTCCCCACAG
ATTCGAAATCTAAACTACACATCACACA

Hybrid promoter P98
Y. lipolytica PGK1 *O. polymorpha CYC1* *O. polymorpha PSH1*

CAGACAGTGACGAGTCATACATTCTCCGTATAATATCGTGTATGTCCAGACGATAGTCGTACTCGTACTC
GTTACTGTAACTACTGTGCGAGTACTCGTGCATGTATCGTAGGTATTGTATGTTCGAGTACATACACATA
CGATACCAAACACTGCCCACTGTTCTGTCATGTTAGATCATGGCGGGGCCACGTGACTTGCATGCAGGTT
TGGCATTGAATATTCAGCGTGGCTACTACAAGTAGTACATACTGTATCAATACGATTGTACATACGGTAC
TGGGTTTCGCAAACAACATGGTAAAATTACAAATGTGACTATTTTTCCACTTTTTATTTTGGTACACTGGC
CTTTCTTTTTGCCATCAGCGCAATTCCCGACACCCGTGCACCGCGACACCCAAAAGTTGTCATTGAAATTT
TCTCGCCGTTTGAGATCGCTTTTGGAAAAAAAGAAAAAAATTACCTGGAAGCTCGTAGAATCCAGGGAG
CCGAGGAATAAACTGGGGGTGCACAGCCTTACACCTTGTTATATATCTCGAGCACACGATCAAAGTGCT
ACAAAACCAGTACGACCACATCAGCGCACAGCATATGCAAAATTTGGACCAGTATCTAAAGAAAATGGC
CCGCTGGAGGGAAATTGTATAAAGATAGACAAATCGACTAAATTTCTAAAACACAAAATAATTTTTTATG
ATCCACATGGATGGAAGTGCTCAAGTTATTGCAATCCTTGGGGGATGAGAACAAGAAGGACTTGATGC
GACGCAGTGTCGAGGTCTTTACTTGTACCATCA

Hybrid promoter P99
Y. lipolytica GPDH *O. polymorpha PFK2* *K. phaffii AOX1*

GTAGGTTGGGTTGGGTGGGAGCACCCCTCCACAGAGTAGAGTCAAACAGCAGCAGCAACATGATAGTT
GGGGGTGTGCGTGTTAAAGGAAAAAAAAAGAAGCTTGGGTTATATTCCCGCTCTATTTAGAGGTTGCG
GGATAGACGCCGACGGAGGGCAATGGCGCCATGGAACCTTGCGGATATGGGGACGCCGCGGCGGACT

GCGTCCGAACCAGCTCCAGCAGCGTTTTTTCCGGGCCATTGAGCCGACTGCGACCCCGCCAACGTGTCTT
GGCGCAACATTCGGGAGCAGCTGGAGCGTATGTGTGAGCGGATGGGGCTTTTGGACGCAAACCAGCC
GGTTTTGGACCACGACCGACTGCTTGTGAACGTGCTGAAGAGCATTGTGGCTGGCTTTTTCGTCAATGCT
GCGCAGCTGAGCCGGTCCGGCGACTCGTACCGGTCGATGAAAAAGAACCAGGCGGTGTGGATGCATCC
GTCGTCGGTGCTGTTCGGCGTGAAGCCGCCGCCGAAGCTGGTGATTTGGATGATTATGCATTGTCTCCA
CATTGTATGCTTCCAAGATTCTGGTGGGAATACTGCTGATAGCCTAACGTTCATGATCAAAATTTAACTG
TTCTAACCCCTACTTGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTATCAT
CATTATTAGCTTACTTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAACGA
CAACTTGAGAAGATCAAAAAACAACTAATTATTCGAAACA

Hybrid promoter P101
Y. lipolytica PGK1 *Y. lipolytica ZWF1* *Y. lipolytica GND2*

CAGACAGTGACGAGTCATACATTCTCCGTATAATATCGTGTATGTCCAGACGATAGTCGTACTCGTACTC
GTTACTGTAACTACTGTGCGAGTACTCGTGCATGTATCGTAGGTATTGTATGTTCGAGTACATACACATA
CGATACCAAACACTGCCCACTGTTCTGTCATGTTAGATCATGGCGGGGCCACGTGACTTGCATGCAGGTT
TGGCATTGAATATTCAGCGTGGCTACTACAAGTAGTACATACTGTATCAATACGATTGTACATACGGTAC
TCACCCTTTGCTACAGTATGTACATACAAGGGCGCAACAGAGCCGAGTTTAATGGTTTGAAACCTAGGT
GGAAGAGGGGCGGGCGAGGTATCGTACTGTGGGTGCGATAGTTCACCAGTCACGCTGGTGGGCCATTC
TCTTAGCACATTTCCCCCCTCCCAAGTCCCCTCAACCCCCAATGTAACCCTCAACCTCCCACAGCTCAGTA
GCACACGTGCAACTAGTTAGTAACAACCCCCCTCCGTCCAGCTTCTCTTTCACACTGCTTAGAGTTCGACT
TCTACTTGAGTGTGGGGAGATTGCTCACAAGTCTGGACTGCCACGGATGACTAAAGTTTGAGCGTTTAA
TCCGCCAAATGACCACACTGTGCATAAACTCGATTGCCAGCGAAATAGAGTTGCTTTACTAAGCACAAA
GTCTGTTGAGTTGGCTGAGACTTGGATTTATAAAACGCTGCAGCGTCCCTCTCCAGACCTTTTCTGCAAC
TTGACATTTTCTTGTTAACGACACCATCACACA

Hybrid promoter P103
K. lactis ENO1 *Y. lipolytica ENO2* *Y. lipolytica GPDH*

GGGACAAAGAAGAATCTTCGTTCTTCTTTCTTGTTCTCAACTTCCCAGCTTCCGTGTGATTACCCTCCGGG
ACAACAGAAAAACTGGCATTCGGTATCCCGGGAATCTGCTGAGAAGGAAAGAAAACGAAAAAAAAATT
GTACATTTGTGTCACATTATGAATTACAGGAAGTCAGAAAACAGGGGGGACATGTCTCGCACATGCATG
TCCATCAGACGAGACATTATGAGACATGCACGCGTGTGAGACTTAGCTACAGAATAAAGCTCGGTAGCG
CCAACAGCGTTGACAAATAGCTCAAGGGCGTGGAGCACAGGGTTTAGGAGGTTTTAATGGGCGAGAAG
GCGCGTAGATGTAGTCTTCCTCGGTCCCATCGGTAATCACGTGTGTGCCGATTTGCAAGACGAAAAGCC
ACGAGAATAAACCGGGAGAGGGGATGGAAGTCCCCGAACAGCAACCAGCCCTTGCCCTCGTGGACATA
ACCTTTCACTTGCCAGAACTCTAAGCGTCACCACGGTATACAAGCGCACGTAGAAGATCCTCGAATTTCA
GACCAGTCACGGCCCCATTCGCCCGCGCAATGGCTCGCCAACGCCCGGTCTTTTGCACCACATCAGGTTA
CCCCAAGCCAAACCTTTGTGTTAAAAAGCTTAACATATTATACCGAACGTAGGTTTGGGCGGGCTTGCTC
CGTCTGTCCAAGGCAACATTTATATAAGGGTCTGCATCGCCGGCTCAATTGAATCTTTTTTCTTCTTCTCTT
CTCTATATTCATTCTTGAATTAAACACACATCAACA

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