Supplementary Materials

Reversal of the β-Oxidation Cycles in *Saccharomyces cerevisiae* for Producing Fuels and Chemicals

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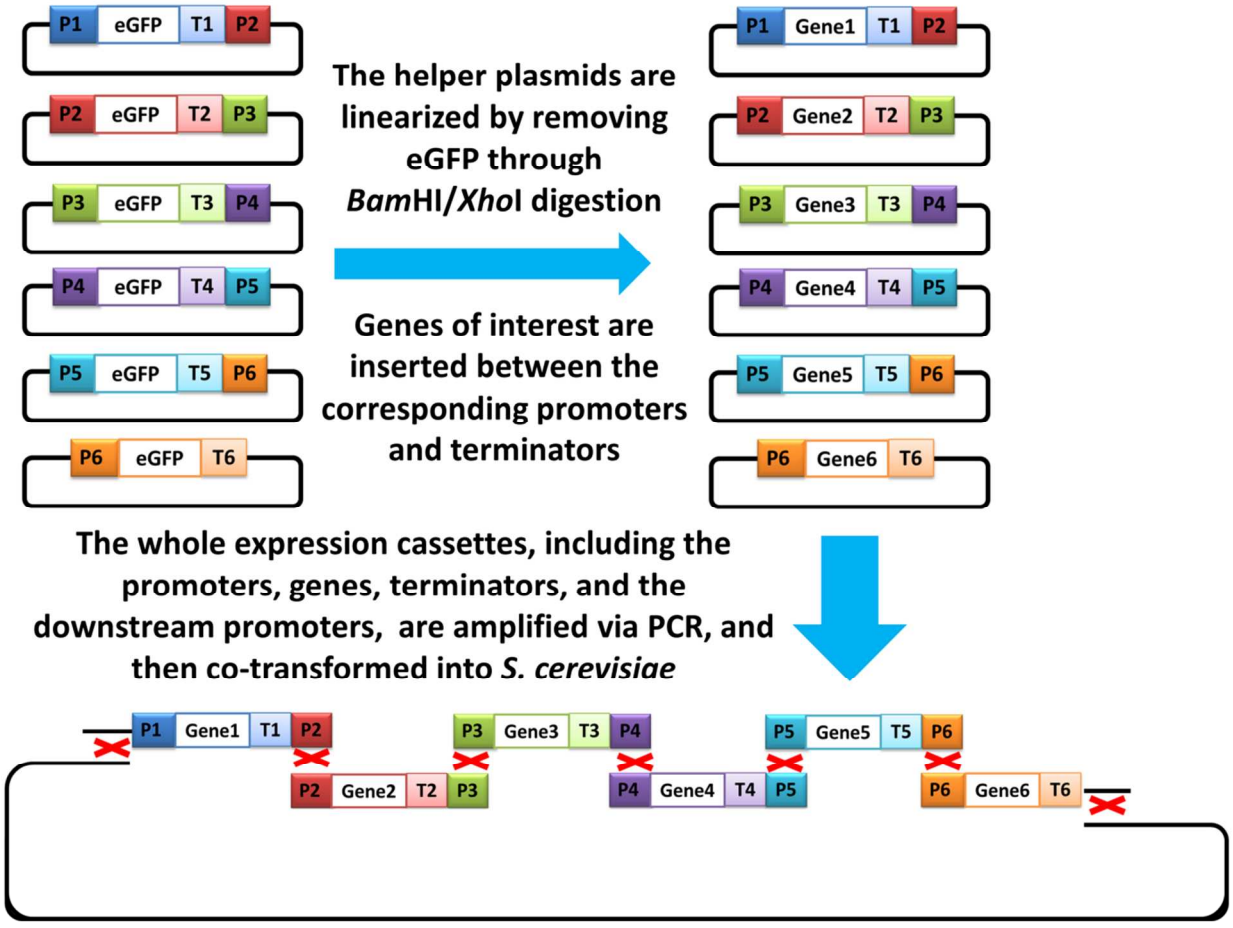
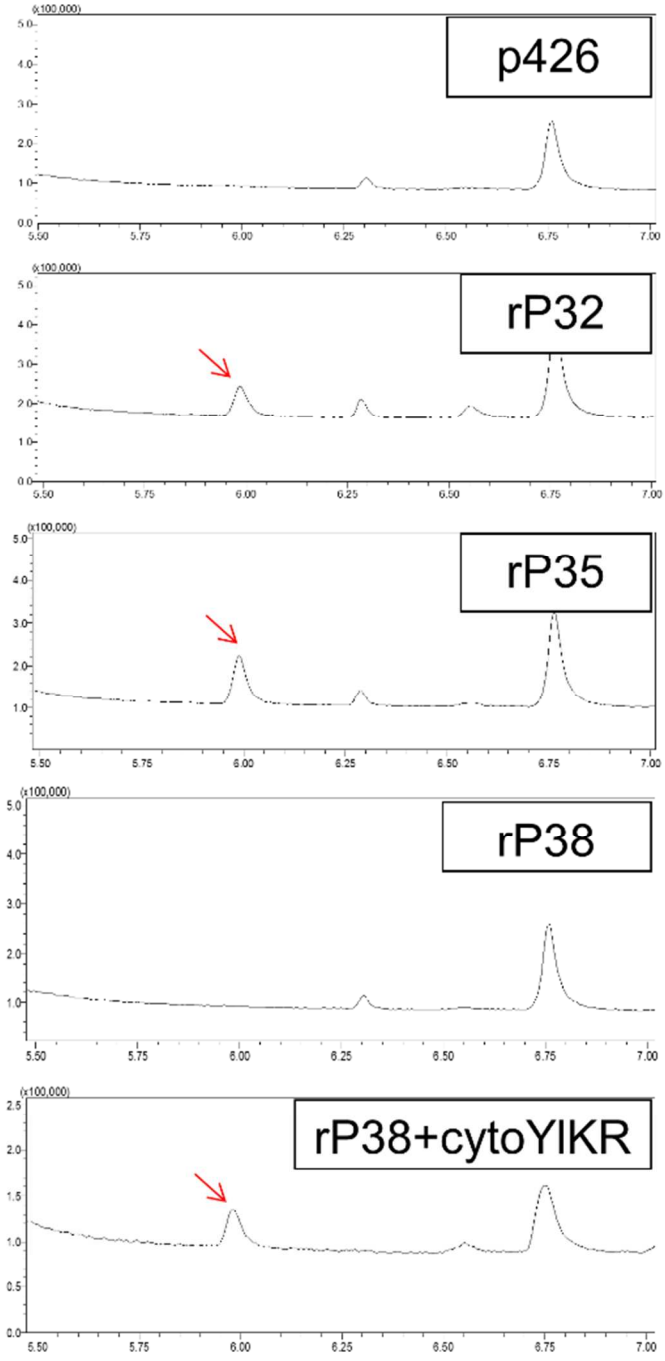
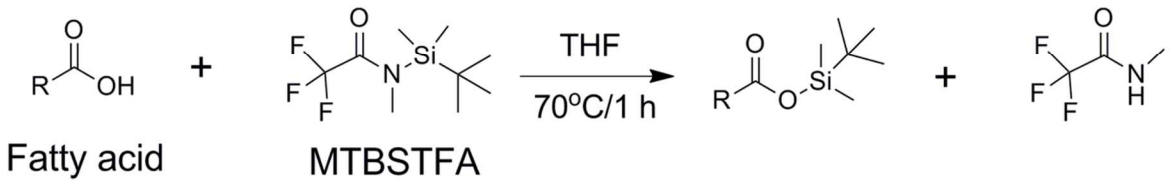
Supplementary Figure S1. Gene cloning and pathway assembly using the helper plasmids strategy. Genes of interest were cloned into the corresponding helper plasmids by replacing the *eGFP* sequence. Then the whole expression cassettes, including the promoters, genes, terminators, and the downstream promoters, were amplified via PCR and co-transformed into *S. cerevisiae*.

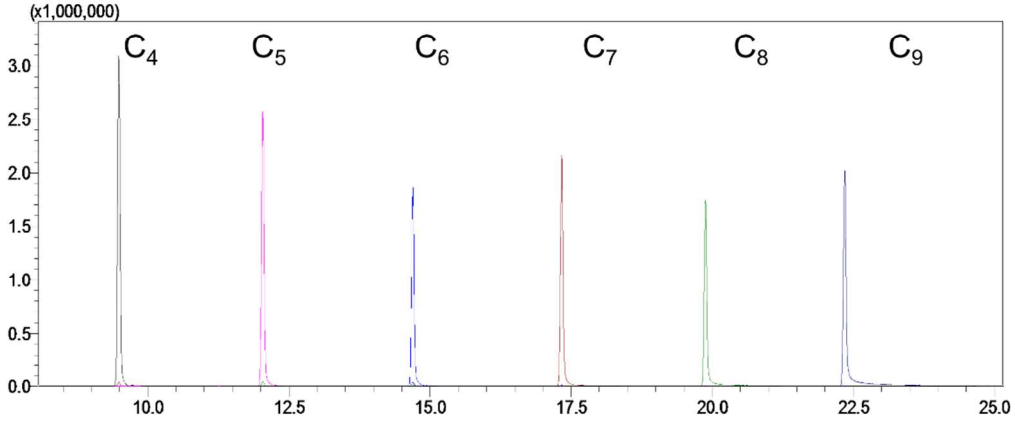


Supplementary Figure S2. GC profiles of *n*-butanol production by the yeast strains containing an empty vector (p426), functional reversed β-oxidation pathways (rP32 and rP35), and a nonfunctional reversed β-oxidation pathway (rP38). The production of *n*-butanol could be restored by co-expressing cytoYlKR with the incomplete reversed β-oxidation pathway (rP38+cytoYlKR, rP38c).

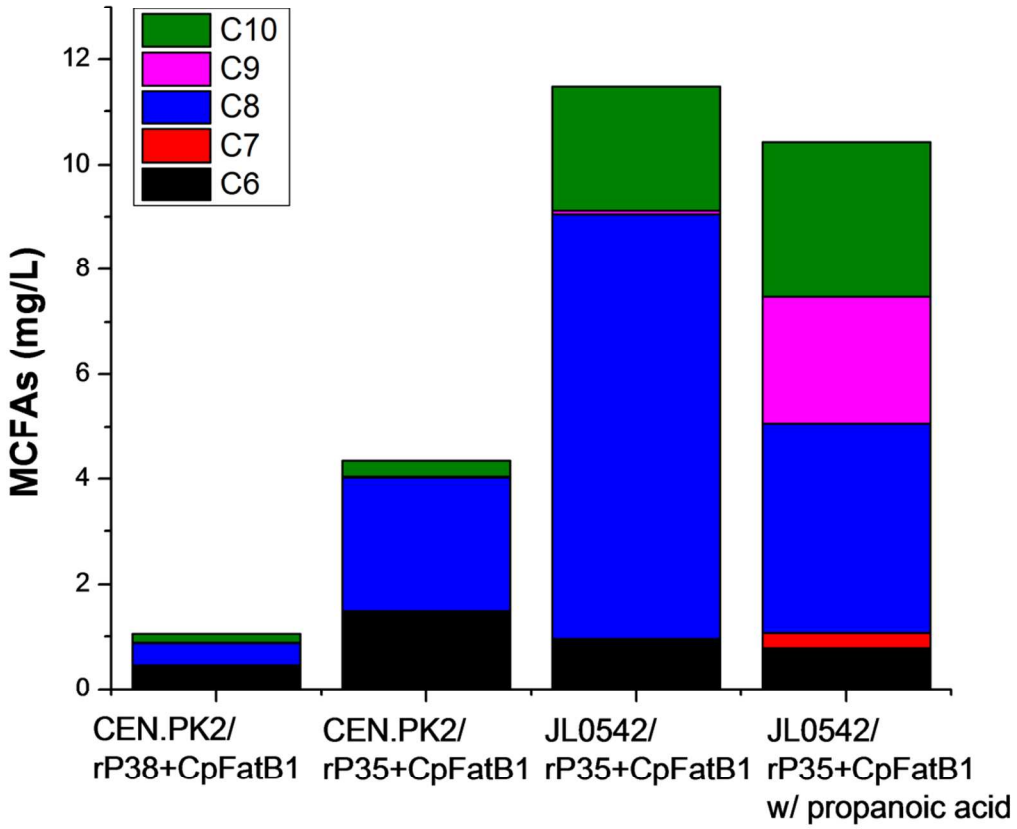


Supplementary Figure S3. The development of a protocol to derivatize and detect short-chain fatty acids by GC-MS. MTBSTFA was chosen as the derivatiation reagents, since the derived fatty acid t-butyldimethylsilyl esters were less volatile and could be more accurately measured. Using the developed GC-MS program, all short- and medium-chain fatty acids could be well separated and detected.

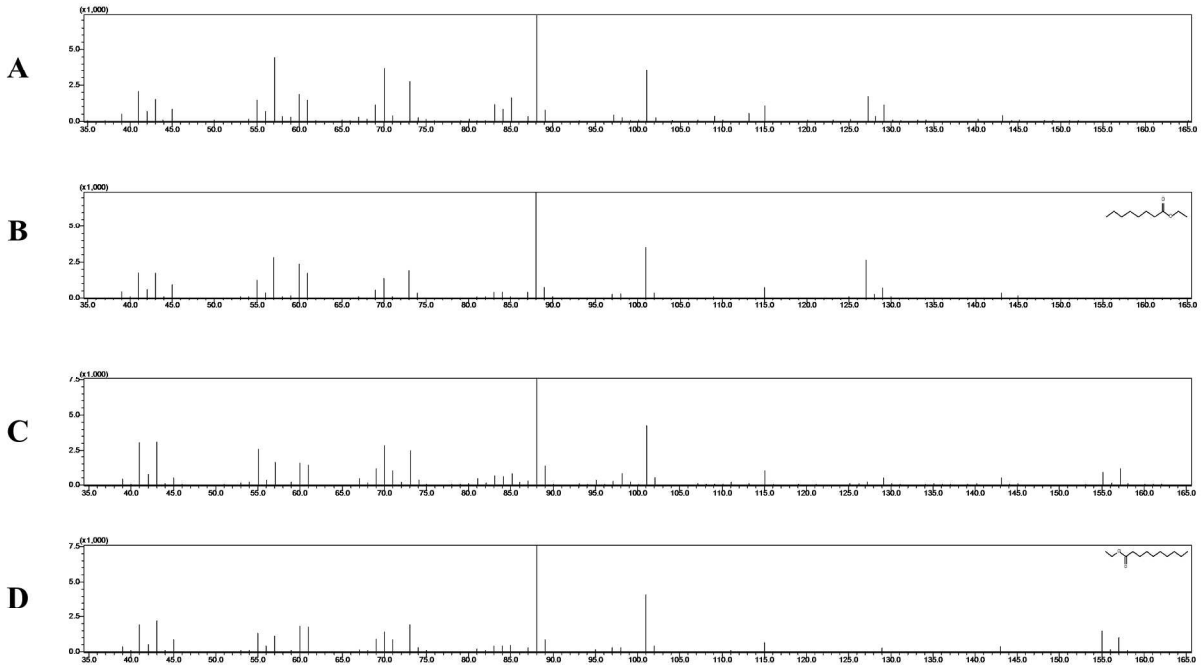




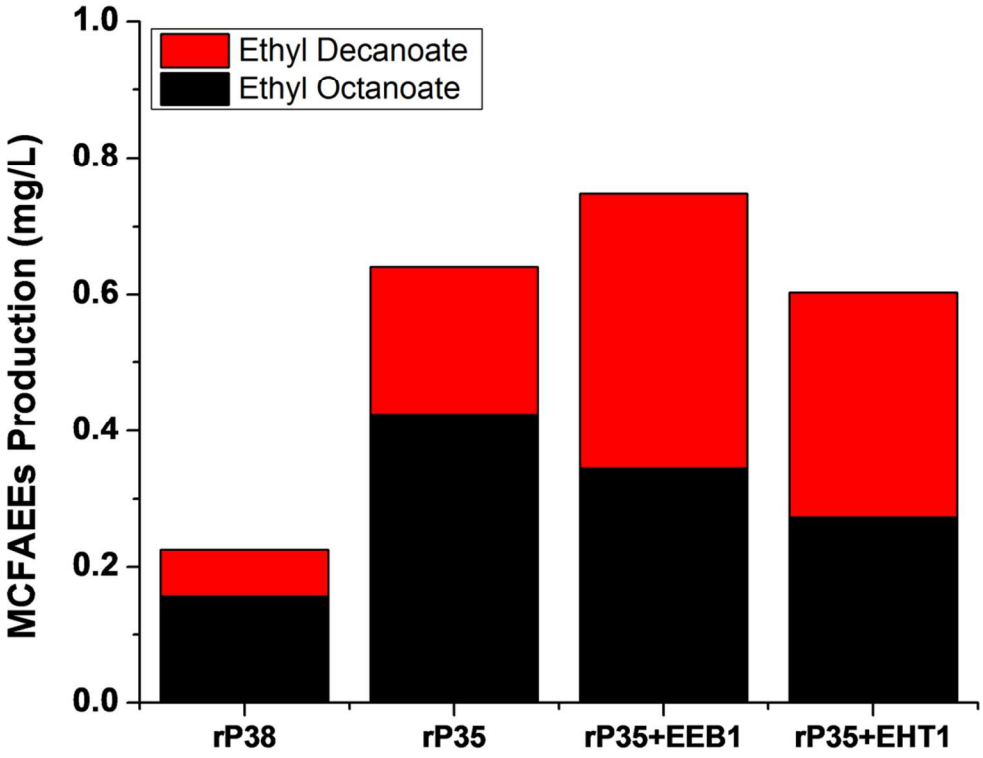
Supplementary Figure S4. Distribution of medium-chain fatty acids produced by yeast strains with or without the reversed β-oxidation pathway.



Supplementary Figure S5. MS fragmentation patterns of the detected MCFAEEs. The MS fragments of ethyl octanoate (A, B) and ethyl decanoate (C, D) were compared between the samples (A, C) and the standards (B, D).



Supplementary Figure S6. Distribution of ethyl octanoate and ethyl decanoate produced by different yeast strains. The strain with functional reversed β-oxidation pathway produced more MCFAEEs. Although the overexpression of EEB1 and EHT1 did not significantly increase the titer of MCFAEEs, the distribution of ethyl octanoate and ethyl decanoate was changed.



Supplementary Table S1. List of oligonucleotides used in this study.

Primer	Sequence (5'-->3')	Application
oJL0201	gcgcgcgtaatacgactcactatagggcgaattgaggcctcatgcgactgggtgagcata	Construction of pH1 (pRS425-GPM1p-eGFP-ADH1t-PYK1p)
oJL0202	ccccggtgaacagctcctcgcccttgctcaccatggatcctttgattgatttgactgtgt	
oJL0203	cctcacgcaaaataacacagtcaaatcaatcaaaggatccatggtgagcaagggcgagga	
oJL0204	cttgaccaaacctctggcgaagaagtccaaagctctcgagttacttgtacagctcgtcca	
oJL0205	gatcactctcggcatggacgagctgtacaagtaactcgagagctttggacttcttcgcca	
oJL0206	ttgtactgagattaatctccaaaatagtagcattcctaggcatgccggtagaggtgtggt	
oJL0207	ggtcgctcttattgaccacacctctaccggcatgcctaggaatgctactattttggagat	
oJL0208	caagcgcgcaattaaccctcactaaagggaacaaaagctgtgtgatgatgttttatttgt	
oJL0437	agcgcgcgtaatacgactcactatagggcgaattgaggccttagtcgtgcaatgtatgac	
oJL0438	ccccggtgaacagctcctcgcccttgctcaccatggatcctattgtaatatgtgtgtttg	
oJL0209	gcgcgcgtaatacgactcactatagggcgaattgcctaggaatgctactattttggagat	Construction of pH2 (pRS425-ADH1t-GPDp-eGFP-CYC1t-ENO2p)
oJL0210	ccccggtgaacagctcctcgcccttgctcaccatggatcctgtgatgatgttttatttgt	
oJL0211	agacaccaatcaaaacaaataaaacatcatcacaggatccatggtgagcaagggcgagga	
oJL0212	gtgaatgtaagcgtgacataactaattacatgatctcgagttacttgtacagctcgtcca	
oJL0213	gatcactctcggcatggacgagctgtacaagtaactcgagatcatgtaattagttatgtc	
oJL0214	aagaaccctttctatacccgcagcgtcgacaccctgcagggcaaattaaagccttcgagc	
oJL0215	aggttttgggacgctcgaaggctttaatttgccctgcagggtgtcgacgctgcgggtata	
oJL0216	caagcgcgcaattaaccctcactaaagggaacaaaagctgtattattgtatgttatagta	
oJL0361	gcgcgcgtaatacgactcactatagggcgaattgcctagg agctttggacttcttcgcca	
oJL0362	ccccggtgaacagctcctcgcccttgctcaccatggatccatccgtcgaaactaagttct	
oJL0217	gcgcgtaatacgactcactatagggcgaattgcctgcagggtgtcgacgctgcgggtata	Construction of pH3 (pRS425-ENO2p-eGFP-PGK1t-TPI1p)
oJL0218	ccccggtgaacagctcctcgcccttgctcaccatggatcctattattgtatgttatagta	
oJL0219	caccaagcaactaatactataacatacaataataggatccatggtgagcaagggcgagga	
oJL0220	aaaaattgatctatcgatttcaattcaattcaatctcgagttacttgtacagctcgtcca	
oJL0221	gatcactctcggcatggacgagctgtacaagtaactcgagattgaattgaattgaaatcg	
oJL0222	tcttccaccaacctgatgggttcctagatatagcggccgccaggaagaatacactatact	
oJL0223	ctctttagatccagtatagtgtattcttcctggcggccgctatatctaggaacccatcag	
oJL0224	caagcgcgcaattaaccctcactaaagggaacaaaagctgttttagtttatgtatgtgtt	
oJL0225	gcgcgtaatacgactcactatagggcgaattggcggccgctatatctaggaacccatcag	Construction of pH4 (pRS425-TPI1p-eGFP-TPI1t-TEF1p)
oJL0226	ccccggtgaacagctcctcgcccttgctcaccatggatccttttagtttatgtatgtgtt	
oJL0227	ctataactacaaaaaacacatacataaactaaaaggatccatggtgagcaagggcgagga	
oJL0228	aagaagataatatttttatataattatattaatcctcgagttacttgtacagctcgtcca	
oJL0229	gatcactctcggcatggacgagctgtacaagtaactcgaggattaatataattatataaa	
oJL0230	gagtaaaaaaggagtagaaacattttgaagctatccgcggtatataacagttgaaatttg	
oJL0231	aagatgttcttatccaaatttcaactgttatataccgcggatagcttcaaaatgtttcta	
oJL0232	caagcgcgcaattaaccctcactaaagggaacaaaagctgtttgtaattaaaacttagat	
oJL0233	gcgcgcgtaatacgactcactatagggcgaattgccgcggatagcttcaaaatgtttcta	Construction of pH5 (pRS425-TEF1p-eGFP-TEF1t)
oJL0234	ccccggtgaacagctcctcgcccttgctcaccatggatcctttgtaattaaaacttagat	
oJL0235	agcatagcaatctaatctaagttttaattacaaaggatccatggtgagcaagggcgagga	
oJL0236	aagatatgcaactagaaaagtcttatcaatctccctcgagttacttgtacagctcgtcca	
oJL0237	gatcactctcggcatggacgagctgtacaagtaactcgagggagattgataagacttttc	
oJL0238	cgcaattaaccctcactaaagggaacaaaagctgcccgggatagcgccgatcaaagtatt	
oJL0421	cagtgagcgcgcgtaatacgactcactatagggcgaattgggagattgataagacttttc	Construct pH6 (pRS425-TEF1t-PGK1p-eGFP-Hxt7t)
oJL0422	ttagagcgtgatcatgaattaataaaagtgttcgcaaaggatcctgttttatatttgttg	
oJL0423	attatctacttttttacaacaaatataaaacaggatcctttgcgaacacttttattaattc	
oJL0424	taggcaccccaggctttacactttatgcttccggctcctatgttgtgtggaattgtgagc	
oJL0671	aagtaattatctacttttttacaacaaatataaaacaggatccatggtgagcaagggcgag	
oJL0672	ttaataaaagtgttcgcaaaaagcttttacttgtacagctcgtccatgccgagagtgatc	
oJL0475	ggttgagtgttgttccagtttggaacaagagtc	Amplify pH1 Cassette
oJL0478	catgccggtagaggtgtggtcaataagag	

oJL0481	agctttggacttcttcgccagaggtttg	Amplify pH2
oJL0482	gcttggtgccacttgtcacatacaattc	Cassette
oJL0483	cctgcagggtgtcgacgctgcgggtatagaaag	Amplify pH3
oJL0484	gtatgtgtttttttgtagttatagatttaagcaag	Cassette
oJL0487	gcggccgctatatctaggaacccatcaggttg	Amplify pH4
oJL0488	gattgctatgctttctttctaatgagcaagaag	Cassette
oJL0489	ccgcggatagcttcaaaatgtttctactc	Amplify pH5
oJL0490	gcgccgatcaaagtatttgttacgacaatatc	Cassette
oJL0491	ggagattgataagacttttctagttgc	Amplify pH6
oJL0492	gggtttcgccacctctgacttgagcgtc	Cassette
oJL0239	tcataacctcacgcaaaataacacagtcaaatcaatcaaa atgtctcagaacgtttacat	Clone THL & KS homologues into pH1
oJL0240	tggagacttgaccaaacctctggcgaagaagtccaaagcttcatatcttttcaatgacaa	
oJL0241	tcataacctcacgcaaaataacacagtcaaatcaatcaaa atgggtaagggtgaatcgaa	
oJL0242	tggagacttgaccaaacctctggcgaagaagtccaaagctctattctttaataaagatgg	
oJL0243	tcataacctcacgcaaaataacacagtcaaatcaatcaaa atgaaaaattgtgtcatcgt	
oJL0244	tggagacttgaccaaacctctggcgaagaagtccaaagctttaattcaaccgttcaatca	
oJL0245	tcataacctcacgcaaaataacacagtcaaatcaatcaaa atgaaagacgttgtgattgt	
oJL0246	tggagacttgaccaaacctctggcgaagaagtccaaagctctattcgtcacgttcaatgg	
oJL0247	tcataacctcacgcaaaataacacagtcaaatcaatcaaa atggaacaggttgtcattgt	
oJL0248	tggagacttgaccaaacctctggcgaagaagtccaaagctttaaacccgctcaaacaccg	
oJL0363	ttttttagttttaaaacaccagaacttagtttcgacggat atgcctggaaatttatcctt	Clone FOX2 (KR+HTD) homologues from different yeast species into pH2
oJL0250	ggcgtgaatgtaagcgtgacataactaattacatgatttagttatccaatacaatgacgt	
oJL0365	ttttttagttttaaaacaccagaacttagtttcgacggat atgctttacaaaggcgacac	
oJL0252	gagggcgtgaatgtaagcgtgacataactaattacatgatttaagccgttttcaggtcgc	
oJL0367	tttagttttaaaacaccagaacttagtttcgacggatatggaaatgacatcagcgtttac	
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oJL0373	ttttttagttttaaaacaccagaacttagtttcgacggatatgtcgtcttcaaatctatc	
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oJL0375	ttttagttttaaaacaccagaacttagtttcgacggatatgagtgaattatcatttaaag	
oJL0376	gagggcgtgaatgtaagcgtgacataactaattacatgatttagtccaaaacaatgacac	
oJL0377	ttttttagttttaaaacaccagaacttagtttcgacggatatgagctcacaacaattatc	
oJL0378	gtgaatgtaagcgtgacataactaattacatgatttaattttctaagacaattttatttc	
oJL0379	tttttagttttaaaacaccagaacttagtttcgacggatatgtcgttagaatttaaggac	
oJL0380	gagggcgtgaatgtaagcgtgacataactaattacatgatttagtccaatacgatcttac	
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oJL0387	tttttagttttaaaacaccagaacttagtttcgacggatatgtcgctaactttcaacgac	
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oJL0389	tttttagttttaaaacaccagaacttagtttcgacggatatgtcccaattaagatttgac	
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oJL0395	ttttagttttaaaacaccagaacttagtttcgacggatatgtcagaaatatcatttaaag	
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oJL0397	ttttagttttaaaacaccagaacttagtttcgacggatatgtcacaattggattttaaag	
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oJL0407	ttttagttttaaaacaccagaacttagtttcgacggatatggccccaataagtttcaaag	
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oJL0409	ttttagttttaaaacaccagaacttagtttcgacggatatgtcggaaatatctttcaaag	
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oJL0411	ttttttagttttaaaacaccagaacttagtttcgacggatatgaagaaggtcgactctctctc	Clone KR from *Yarrowia lipolytica* into pH2
oJL0412	gagggcgtgaatgtaagcgtgacataactaattacatgatttagtagtcatagaagccc	
oJL0425	aaggaagtaattatctacttttttacaacaaatataaaacaatgcctctcgattactccgc	Clone HTD from *Yarrowia lipolytica* into pH6
oJL0426	aattagagcgtgatcatgaattaataaaagtgttcgcaaatcacaacttctcgtaagtag	
oJL0261	tcataacaccaagcaactaatactataacatacaataata atgtcgtcctcagctcatca	Clone TER homologues into pH3
oJL0262	aaagaaaaaaattgatctatcgatttcaattcaattcaatttaccattctaaaacaacca	
oJL0263	tcataacaccaagcaactaatactataacatacaataata atgaaatgtactataccaga	
oJL0264	aaagaaaaaaattgatctatcgatttcaattcaattcaatctattgtggtatttcaagaa	
oJL0265	tcataacaccaagcaactaatactataacatacaataata atgaatacagcaaacacttt	
oJL0266	gaaaaaaattgatctatcgatttcaattcaattcaatttaatacaactcatgtccaaaat	
oJL0267	tcataacaccaagcaactaatactataacatacaataata atgtccgcctcgattccaga	
oJL0268	aaagaaaaaaattgatctatcgatttcaattcaattcaatttatttcaagacggcaacca	
oJL0269	tcataacaccaagcaactaatactataacatacaataata atgcaagttgcaattccaga	
oJL0270	aaagaaaaaaattgatctatcgatttcaattcaattcaatctagtttaatacggcaacga	
oJL0271	tcataacaccaagcaactaatactataacatacaataata atgaaggctgtcgtcattga	
oJL0272	aaagaaaaaaattgatctatcgatttcaattcaattcaatctagtttaatacggcaacta	
oJL0273	tcataacaccaagcaactaatactataacatacaataata atggatttttctttaactga	
oJL0274	aaagaaaaaaattgatctatcgatttcaattcaattcaatttatttgttctgatagtctt	
oJL0275	tcataacaccaagcaactaatactataacatacaataataatgggttttctttccggtaa	
oJL0276	aaagaaaaaaattgatctatcgatttcaattcaattcaatttatttcagttcgagttcgt	
oJL0277	tcataacaccaagcaactaatactataacatacaataata atggccatgttcaccactac	
oJL0278	aaagaaaaaaattgatctatcgatttcaattcaattcaatttactgctgagctgcgctcg	
oJL0279	taacaccaagcaactaatactataacatacaataataatgattgtaaaaccaatggttag	
oJL0280	aagaaaaaaattgatctatcgatttcaattcaattcaatttaaatcctgtcgaacctttc	
oJL0305	ttaaatctataactacaaaaaacacatacataaactaaaa atgaatcaacaggatattga	Clone EcEutE into pH4
oJL0306	aaagaaaagaagataatatttttatataattatattaatcttaaacaatgcgaaacgcat	
oJL0557	aaagaaagcatagcaatctaatctaagttttaattacaaaatggttgatttcgaatattc	Clone CaBdhB into pH5
oJL0558	aaaagatatgcaactagaaaagtcttatcaatctccttacacagatttttgaatatttg	
oJL0795	gttcgcggatcc atgtcagaagtttccaaatggccag	Clone EEB1 and EHT1 into pH5
oJL0796	cacccgctcgag tcatacgactaattcatcaaacttag	
oJL0797	gttcgcggatcc atgtttcgctcgggttactatc	
oJL0798	cacccgctcgag ttataaaactaactcatcaaag	
oJL0537	catcaagaacaaacaagctcaacttgtc	Sequencing primers for genes cloned into Helper plasmids
oJL0538	caagatatcattaaaaatataaaattag	
oJL0539	catatttcttgtcatattcctttctc	
oJL0540	cttttcgtaaatttctggcaaggtag	
oJL0541	cttaacttgtttattattctctcttgtttc	
oJL0542	cttcaggttgtctaactccttcctttc	

oJL0543	gtatcttttcttcccttgtctcaatc
oJL0544	ctattattttagcgtaaaggatgggg
oJL0545	catttactattttcccttcttacg
oJL0546	caatataaaaaagctttccgtagtcatc
oJL0547	gtttcatttttcttgttctattacaac
oJL0548	ccagactatatataaggataaattac
oJL0549	ggtaggtattgattgtaattctgtaaatc
oJL0550	ctaattcgtagtttttcaagttcttagatgc
oJL0551	cctttcttaattctgttgtaattaccttcc

Supplementary Table S2. DNA sequences of the gBlock fragments to clone CpFatB1.The homology arms at both 5' and 3' end were underlined.

gBlocks	Sequence (5'-->3')
CpFatB1-Fg1	tgctcattagaaagaaagcatagcaatctaatctaagttttaattacaaaatggtggctgctgcagcaagttctgcatgcttccctgttccatcccc aggagcctcccctaaacctggtaagttaggcaactggtcatcgagtttgagcccttccttgaagcccaagtcaatccccaatggcggatttcag gttaaggcaaatgccagtgcgcatcctaaggctaacggttctgcagtaactctaaagtctggcagcctcaacactcaggaggacactttgtcgt cgtcccctcctcccgggctttttttaaccagttgcctgattggagtatgcttctgactgcaatcacaaccgtcttcgtggcaccagagaagcggt ggactatgtttgataggaaatctaagaggcctaacatgctcatggactcgtttgggttggagagagttgttcaggatgggctcgtgttcagaca gagtttttcgattaggtcttatgaaatatgcgctgatcgaacagcctctatagagacggtgatgaaccacgtccaggaaacatcactcaatcaat gtaagagtataggtcttctcgatgacggctttggtcgtagtcctgagatgtgtaaaagggacctcatttgggtggttacaagaatgaagataatg gtgaatcgctatccaacttggggcgatactatcgag
CpFatB1-Fg2	gaatgaagataatggtgaatcgctatccaacttggggcgatactatcgaggtcagtacctggctctctcaatcggggaaaatcggtatgggtc gcgattggctaataagtgattgcaacacaggagaaattcttgtaagagcaacgagtgtgtatgccatgatgaatcaaaagacgagaagattctc aaaactcccacacgaggttcgccaggaatttgcgcctcattttctggactctcctcctgccattgaagacaacgacggtaaattgcagaagtttg atgtgaagactggtgattccattcgcaagggtctaactccggggtggtatgacttggatgtcaatcagcacgtaagcaacgtgaagtacattgg gtggattctcgagagtatgccaacagaagttttggagactcaggagctatgttctctcacccttgaatataggcgggaatgcggaagggacag tgtgctggagtccgtgacctctatggatccctcaaaagttggagaccggtttcagtaccggcaccttctgcggcttgaggatggggctgatatc atgaagggaagaactgagtggcggccgaagaatgcaggaactaacggggcgatatcaacaggaaagacttgaggagattgataagacttttt ctagttgcatatcttttatatttaaatctta