

Additional file1

Table 2 Plasmids used in this work.

Plasmid	Relevant characteristics	Source or reference
pUG6	DNA-template for amplification of <i>loxP-kanMX-loxP</i> gene resistance marker gene	[49]
pCZ1	DNA-template for amplification of <i>loxP-hphNT1-loxP</i> gene resistance marker gene	[59]
pCZ2	DNA-template for amplification of <i>loxP-natNT2-loxP</i> gene resistance marker gene	[59]
pSH47	Cre-recombinase under control of <i>GALI</i> promoter; <i>URA3</i> marker gene	[49]
p423H7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>HIS3</i> marker gene	[60]
p424H7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>TRP1</i> marker gene	[60]
p426H7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>URA3</i> marker gene	[60]
pRS42K	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>kanMX</i> marker gene	[61]
pRS42N	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>natNT2</i> marker gene	[61]
pRS42KH7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>kanMX</i> marker gene	This work
pRS42HH7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>FBA1</i> terminator; <i>hphNT1</i> marker gene	This work
pRS42NH7	2 μ -plasmid with shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator; <i>natNT2</i> marker gene	This work
p424H7-Aro10	2 μ -plasmid; <i>ARO10</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>TRP1</i> marker gene	This work
p424H7-kivD	2 μ -plasmid expressed with a N-terminal, 6-fold Histidin tagged, version of <i>kivD</i> of <i>Lactococcus lactis</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>TRP1</i> marker gene	This work
p426H7-Adh1	2 μ -plasmid; <i>ADH1</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-Adh2	2 μ -plasmid; <i>ADH2</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-Adh3	2 μ -plasmid; <i>ADH3</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-Adh4	2 μ -plasmid; <i>ADH4</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work

p426H7-Adh6	2 μ -plasmid; <i>ADH6</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-Sfa1	2 μ -plasmid; <i>SFA1</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2 Δ N54tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 54 amino acids of <i>S. cerevisiae</i> <i>ILV2</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2 Δ N85tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 85 amino acids of <i>S. cerevisiae</i> <i>ILV2</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2wttag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, full length version of <i>S. cerevisiae</i> <i>ILV2</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
pRS42KH7-ILV3 Δ N19tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 19 amino acids of <i>S. cerevisiae</i> <i>ILV3</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3 Δ N34tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 34 amino acids of <i>S. cerevisiae</i> <i>ILV3</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3 Δ N42tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 42 amino acids of <i>S. cerevisiae</i> <i>ILV3</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3 Δ N50tag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 50 amino acids of <i>S. cerevisiae</i> <i>ILV3</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3 Δ N19DEtag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 19 amino acids of <i>S. cerevisiae</i> <i>ILV3</i> with an additional insertion of aspartate and glutamate under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3wttag	2 μ -plasmid expressed with a C-terminal, 6-fold Histidin-tagged, full length version of <i>S. cerevisiae</i> <i>ILV3</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work

p426H7-ILV5ΔN48tag	2μ-plasmid expressed with a C-terminal, 6-fold Histidin-tagged, N-terminal truncated version of 48 amino acids of <i>S. cerevisiae ILV5</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV5wttag	2μ-plasmid expressed with a C-terminal, 6-fold Histidin-tagged, full length version of <i>S. cerevisiae ILV5</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2ΔN54	2μ-plasmid; truncated version of <i>S. cerevisiae ILV2</i> lacking N-terminal 54 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2ΔN85	2μ-plasmid; truncated version of <i>S. cerevisiae ILV2</i> lacking N-terminal 85 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
p426H7-ILV2wt	2μ-plasmid; <i>ILV2</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>URA3</i> marker gene	This work
pRS42KH7-ILV3ΔN19	2μ-plasmid; truncated version of <i>S. cerevisiae ILV3</i> lacking N-terminal 19 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3ΔN34	2μ-plasmid; truncated version of <i>S. cerevisiae ILV3</i> lacking N-terminal 34 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3ΔN42	2μ-plasmid; truncated version of <i>S. cerevisiae ILV3</i> lacking N-terminal 42 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3ΔN50	2μ-plasmid; truncated version of <i>S. cerevisiae ILV3</i> lacking N-terminal 50 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3ΔN19DE	2μ-plasmid; truncated version of <i>S. cerevisiae ILV3</i> lacking N-terminal 19 amino acids with inserted glutamate and aspartate under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
pRS42KH7-ILV3wt	2μ-plasmid; <i>ILV3</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>kanMX</i> marker gene	This work
p423H7-ILV5ΔN48	2μ-plasmid; truncated version of <i>S. cerevisiae ILV5</i> lacking N-terminal 48 amino acids under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>HIS3</i> marker gene	This work
p423H7-ILV5wt	2μ-plasmid; <i>ILV5</i> of <i>S. cerevisiae</i> under control of shortened <i>HXT7</i> promoter and <i>CYC1</i> terminator, <i>HIS3</i>	This work

	marker gene	
pRS42NH7-ARO10	2 μ -plasmid; <i>S. cerevisiae</i> ARO10 under control of shortened HXT7 promoter and CYC1 terminator, natNT2 marker gene	This work
pRS42HH7-ADH2	2 μ -plasmid; <i>S. cerevisiae</i> ARO10 under control of shortened HXT7 promoter and FBA1 terminator, hphNT1 marker gene	This work
p425-synthILV235	2 μ -plasmid with integrative ILV-cassette which contains truncated ORFs of codon-optimized ILV2 Δ N54, codon-optimized ILV5 Δ N48 and codon-optimized ILV3 Δ N19 of <i>S. cerevisiae</i> ; codon-optimized ILV2 Δ N54 under control of shortened HXT7 promoter and CYC1 terminator, codon-optimized ILV5 Δ N48 under control of FBA1 promoter and PGK1 terminator, codon-optimized ILV3 Δ N19 under control of PFK1 promoter and FBA1 terminator, loxP-kanMX-loxP resistance gene, flanked of 369bp and 385bp homologous to FMO1-locus, respectively, LEU2 marker gene in 2 μ -plasmid; capability of integration into chromosomVIII of codon-optimized ILV-cassette through <i>in vivo</i> -recombination after restriction by AscI/PacI	This work

Table 3 Oligonucleotides used in this work.

Oligonucleotides	Sequence	Used for construction of	
Ilv2-wt-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGATCA GACAATCTACGCTAA	p426H7-ILV2wt, ILV2wttag	p426H7-
ILV2 Δ N54-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGCCA GAGCCTGCTCCAAG	p426H7-ILV2 Δ N54, ILV2 Δ N54tag	p426H7-
ILV2 Δ N85-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGATA CCTCTTTCGTCCG	p426H7-ILV2 Δ N85, ILV2 Δ N85tag	p426H7-
ILV2-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTCAGTGCTT ACCGCCTGTACG	p426H7-wtILV2, ILV2 Δ N54, p426H7-ILV2 Δ N85	p426H7-
ILV2-C-Tag-r	AACTAATTACATGACTCGAGTCA GTGATGGTGTGGTGTGGTGCT TACCGCCTGTACGC	p426H7-ILV2wttag, ILV2 Δ N54tag, ILV2 Δ N85tag	p426H7- p426H7-
Ilv5-wt-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTTG AGAACTCAAGCCGC	p423H7-ILV5wt, ILV5wttag	p426H7-
ILV5 Δ N48-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGAAG CAAATCAACTTCGGT	p423H7-ILV5 Δ N48, ILV5 Δ N48tag	p426H7-
ILV5-r	GAATGTAAGCGTGACATAACTAA	p423H7-ILV5wt,	p423H7-

	TTACATGACTCGAGTTATTGGTTT TCTGGTCTCAAC	ILV5ΔN48	
ILV5-C-Tag-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTAGTGATG GTGATGGTGATGTTGGTTTTCTG GTCTCAAC	p426H7-ILV5wttag, ILV5ΔN48tag	p426H7-
Ilv3-wt-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGGC TTGTAAACGAAAGTTG	pRS42KH7-ILV3wt, pRS42KH7-ILV3wttag	
ILV3ΔN19DE-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGAT GAGGCAAAGAAGCTC	pRS42KH7-ILV3ΔN19DE, pRS42KH7-ILV3ΔN19DEtag	
ILV3ΔN19-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGCA AAGAAGCTCAACAAG	pRS42KH7-ILV3ΔN19, pRS42KH7-ILV3ΔN19tag	
ILV3ΔN34-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGGC CAAGGTGCGTCCCAG	pRS42KH7-ILV3ΔN34, pRS42KH7-ILV3ΔN34tag	
ILV3ΔN42-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGCTTT ATGCCACCGTTTTT	pRS42KH7-ILV3ΔN42, pRS42KH7-ILV3ΔN42tag	
ILV3ΔN50-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGAA GATTTCAAGAAGCCT	pRS42KH7-ILV3ΔN50, pRS42KH7-ILV3ΔN50tag	
ILV3-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTCAAGCATC TAAAACACAACCG	pRS42KH7-ILV3wt, pRS42KH7-ILV3ΔN19DE, pRS42KH7-ILV3ΔN19, pRS42KH7-ILV3ΔN34, pRS42KH7-ILV3ΔN42, pRS42KH7-ILV3ΔN50	
ILV3-C-Tag-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTCAGTGATG GTGATGGTGATGAGCATCTAAAA CACAACCGTTG	pRS42KH7-ILV3wttag, pRS42KH7-ILV3ΔN19DEtag, pRS42KH7-ILV3ΔN19tag, pRS42KH7-ILV3ΔN34tag, pRS42KH7-ILV3ΔN42tag, pRS42KH7-ILV3ΔN50tag	
ARO10-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGGCA CCTGTTACAATTGAAAA	p424H7-Aro10, ARO10	pRS42NH7-
ARO10-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGCTATTTTTTA TTTCTTTTAAGTGCC	p424H7-Aro10, ARO1	pRS42NH7-
KivD-f	TTTAATCAAAAAGTTAACATGCA TCACCATCACCATCACTATACAGT AGGAGATTACC	p424H7-kivD	
KivD-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTATGATTA TTTTGTTTCAGCAAATA	p424H7-kivD	
ADH2-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCTA	pRS42HH7-ADH2	

ADH2-r	TTCCAGAAACTCA CAATACTCATTAATAAACTATATC AATTAATTTGAATTAAGTTATTTA GAAGTGTCAACAACGTATCTACC AGC	pRS42HH7-ADH2
ADH1-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCTA TCCCAGAAACTCA	p426H7-Adh1
ADH1-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTATTTAGA AGTGTCAACAA	p426H7-Adh1
ADH2-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCTA TTCCAGAAACTCA	p426H7-Adh2
ADH2-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTATTTAGA AGTGTCAACAA	p426H7-Adh2
ADH3-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTTG AGAACGTCAACATT	p426H7-Adh3
ADH3-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTATTTACTA GTATCGACGA	p426H7-Adh3
ADH4-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCTT CCGTTACTGGGT	p426H7-Adh4
ADH4-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGTTAATATTCA TAGGCTTTCT	p426H7-Adh4
ADH6-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCTT ATCCTGAGAAATT	p426H7-Adh6
ADH6-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGCTAGTCTGA AAATTCTTTGT	p426H7-Adh6
SFA1-f	AACACAAAAACAAAAAGTTTTT TTAATTTTAATCAAAAAATGTCC GCCGCTACTGTTGG	p426H7-Sfa1
SFA1-r	GAATGTAAGCGTGACATAACTAA TTACATGACTCGAGCTATTTTATT TCATCAGACT	p426H7-Sfa1
FM01-1_1	CAAGCGCGCAATTAACCCTCACT AAAGGGAACAAAAGCTGTTAAT TAACGTAAAAAGGAATGTAAGT	p425-synthILV235
FM01-1_2	CCGATTTCACTTTCTCATCCTTAT ATTTTTCCTGTGCGGATTTTCCAG ATTC	p425-synthILV235
FM01-2_1	GTATGCTATACGAAGTTATTAGGT GATATCAGATCCACTAGTGGAGG CCATCCTTTTAAAGATC	p425-synthILV235

FMO1-2_2	GGAACCCTAAAGGGAGCCCCCG ATTTAGAGCTTGACGGCGCGCCG ATTTTCCTCAGTCCCTTAGG	p425-synthILV235
loxP-1	CGAAAATTCTGCGTTCGTAAAG CTTTCGAGAAGGATATTATTGCT GAAGCTTCGTACGCTGC	p425-synthILV235
loxP-2	CCACTAGTGGATCTGATATC	p425-synthILV235
FMO1-PFK1p-f	GTAAAAAAGAAACTTTC TTTTTGAATCTGGAAAATCCGCA CAGGAAAATATAAG GATGAGAAAGTGAAATC	p425-synthILV235
PFK1p- Ilv3ΔN48-r Ilv3ΔN48-f	CTTTGATATGATTTTGTTCAGAT TTTTTATA AGCTTTTATATAAAAAATCTGAA ACAAAATCATATCAAAGATGGCT AAGAAGTTGAACAA	p425-synthILV235
Ilv3ΔN48-r	AATACTCATTAAAAACTATATCA ATTAATTTGAATTAAGCGT CCAAAACACAAC	p425-synthILV235
Ilv3ΔN48- FBA1t-f	GGTTTCTAACGCTTCTAACGGTT GTGTTTTGGACGCTTAAGTTAAT TCAAATTAATTGATATAGTTTTTT AATGAG	p425-synthILV235
FBA1t-HXT7p- r	CAGAAGAACACGCAGGGGCCCG AAATTGTTCTACGAGCATGAGC TATCAAAAACGATAGATCGATTA G	p425-synthILV235
HXT7p-f HXT7p- Ilv2ΔN54-r	GCTCGTAGGAACAATTCGGGCC TTTTTGATTAATAAAAAAAC TTTTTGTTTT	p425-synthILV235
HXT7p- Ilv2ΔN54-f	AAACACAAAAACAAAAAGTTTT TTAATTTAATCAAAAAATGCC AGAACCAGCTCCATC	p425-synthILV235
Ilv2ΔN54- CYC1t-r	CGTGAATGTAAGCGTGACATAAC TAATTACATGACTCGAGTTAGTG CTTACCACCGGT	p425-synthILV235
CYC1t-f	CTCGAGTCATGTAATTAGTTATGT CACGC	p425-synthILV235
CYC1-r CYC1t-FBA1p- f	AAATTAAGCCTTCGAGCGTCC TTGCTTGAGAAGGTTTTGGGACG CTCGAAGGCTTTAATTTGGGTC ATTACGTAAATAAT	p425-synthILV235
FBA1p-r	TTTGAATATGTACTTGGTTAT GGTTATAT	p425-synthILV235
FBA1p- Ilv5ΔN48-f	TTTGTCATATATAACCATAACCAA GTAATACATATTCAAAATGAAGC AAATTAACCTTCGG	p425-synthILV235
Ilv5ΔN48- PGK1t-r	GAGAAAAGAAAAAATTGATCT ATCGATTTCAATTCATTTCAATTT ATTGGTTTTCTGGTCTCAACTTTC	p425-synthILV235

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PGK1-f	ATTGAATTGAATTGAAATCGATA GATCAATT	p425-synthILV235
PGK1-r	AAATAATATCCTTCTCGAAAGCT TTAACGA	p425-synthILV235
Del-ilv2-f	GAGCTAAGAGGAGATAAATACA ACAGAATCAATTTTCAATTCGTA CGCTGCAGGTCGAC	Isoy8, Isoy16
Del-ilv2-r	TTTTTACTGAAAATGCTTTTGAA ATAAATGTTTTTGAAATGCATAG GCCACTAGTGGATC	Isoy8, Isoy16
Del-ilv3-f	TTCTTGATTTTTTTTGTAACAGC CAAGAAAAAAGTAGAGTTCGTA CGCTGCAGGTCGAC	Isoy10, Isoy16
Del-ilv3-r	ATCTCTATATATATATTCATCGATT GGGGCCTATAATGCAGCATAGGC CACTAGTGGATC	Isoy10, Isoy16
Del-ilv5-f	ATTTTTTTACCCTACCAGCAATAT AAGTAAAAAATAAACTTCGTAC GCTGCAGGTCGAC	Isoy12, Isoy16
Del-ilv5-r	CAAGAGAAAAAGTTTCCAGCAC TTGATATTATTTTCTCTGCATAG GCCACTAGTGGATC	Isoy12, Isoy16
