Enhanced production of isobutyl and isoamyl acetate using Yarrowia lipolytica

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Table S1. Plasmids used in this study

Plasmids	**Characteristic**	**Reference**
pCRISPRyl	Vector for expressing CAS9 and sgRNA in *Y. lipolytica*: empty sgRNA target	Addgene (#70007)
pCRISPRyl *_A08*	pCRISPRyl derivative, *A08* sgRNA target	28
pCRISPRyl *_d17*	pCRISPRyl derivative, *d17* sgRNA target	28
pCRISPRyl *_c18*	pCRISPRyl derivative, *c18* sgRNA target	28
pCRISPRyl *_xyr11*	pCRISPRyl derivative, *xyr11* sgRNA target	28
pCRISPRyl *_xyr6*	pCRISPRyl derivative, *xyr6* sgRNA target	This study
pCRISPRyl *_dga1*	pCRISPRyl derivative, *dga1* sgRNA target	28
pCRISPRyl *_dga2*	pCRISPRyl derivative, *dga2* sgRNA target	This study
pCRISPRyl *_lro1*	pCRISPRyl derivative, *lro1* sgRNA target	28
pCRISPRyl *_tgl4*	pCRISPRyl derivative, *tgl4* sgRNA target	This study
pCRISPRyl *_oil1*	pCRISPRyl derivative, *oil1* sgRNA target	This study
pHR_A08_hrGFP	Vector for constructing a gene expression casette under UAS1B8-TEF promoter control	Addgene (#84615)
pHR_XYR11*_eat1*	Vector for constructing an *eat1* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR6*_atf1*	Vector for constructing an *atf1* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR*_atf2*	Vector for constructing an *atf2* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR11*_eht1*	Vector for constructing an *eht1* expression cassette under UAS1B8-TEF promoter control	This study

pHR_XYR6_*Wan*	Vector for constructing an *eat1* from *Wickerhamomyces anomalus* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR11_*Wci*	Vector for constructing an *eat1* from *Wickerhamomyces ciferrii* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR6_*Kma*	Vector for constructing an *eat1* from *Kluyveromyces marxianus* expression cassette under UAS1B8-TEF promoter control	This study
pHR_A08_*atf1*	Vector for constructing an *atf1* expression cassette under UAS1B8-TEF promoter control	This study
pHR_XYR11_*atf1*	Vector for constructing na *atf1* expression cassette under UAS1B8-TEF promoter control	This study
pHR_ *pex10*	Vector for constructing a *pex10* expression cassette under UAS1B8-TEF promoter control	28
pHR_ *por1*	Vector for constructing a *por1* expression casette under UAS1B8-TEF promoter control	28
pHR_ *acs1*	Vector for constructing an *acs1* expression cassette under UAS1B8-TEF promoter control	28
pHR_ *pdc2*	Vector for constructing a *pdc2* expression cassette under UAS1B8-TEF promoter control	28
pHR_ *ald5*	Vector for constructing a *ald5* expression casette under UAS1B8-TEF promoter control	28

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Table S2. Primers used in this study

Primer Name	**Sequence**
fw-pHR-inv	GCTAGCCTCATGTAATTAGT
rev-pHR-inv	CTTTGAATGATTCTTATACT
fw-pHR-eat1-insert	AAGAATCATTCAAAGATGTCTCGACTGGCCCACAA
rev-pHR-eat1-insert	TTACATGAGGCTAGCTTACTCGAAGTCCAGGGTTT
fw-pHR-atf1-insert	AATCATTCAAAGATGAACGAGATCGACGAG
rev-pHR-atf1-insert	ATGAGGCTAGCTTAGGGGCCCAGCAGCAG
fw-pHR-atf2-1-insert	AAGAATCATTCAAAGATGGAAGATATCGAGGGCTA
rev-pHR-atf2-1-insert	AGAGGTCTTGAAGTTGCAGTGCTCGTAGATGAAGGTGGTC
fw-pHR-atf2-1-insert	GACCACCTTCATCTACGAGCACTGCAACTTCAAGACCTCT
rev-pHR-atf2-1-insert	TTACATGAGGCTAGCTTACAGAGAGGCGAACTCGC
fw-pHR-eht1-1-insert	AAGAATCATTCAAAGATGTCTGAGGTGTCTAAGTG
rev-pHR-eht1-1-insert	TCTCTCGCTGCAGAAACTCTCGGATGTCCATAGTGTGGTA

fw-pHR-eht1-2-insert	TACCACACTATGGACATCCGAGAGTTTCTGCAGCGAGAGA
rev-pHR-eht1-2-insert	TTACATGAGGCTAGCTTACACCACCAGCTCGTCAA
fw-Cas9-xyr6-inv	GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC
rev-Cas9-xyr6-inv	TTCTAGCTCTAAAACCCTTGTACTCCTGTGACAATACGTCAACCTGCGCCGACCCGG
fw-Avr2-xyr6	GGCTTTAATTTGCCCTAGGGAGTGCACCGGAGCTGAGCCTTCTATTC
rev-Spe1-xyr6	GAATTCGGGTACCACTAGTGCCACTCGATCTCCCACCTTGAGGTC
fw-xyr6-1	TCGTCTTCACCTCGAGATCAACACCTTAAACCGGTACCCAATGCTC
rev-xyr6-2	GCGTACCATGGGATCCCCGAGTACAACACCCACTTTCTGACTAAGCTG
fw-xyr6-3	GGCGAATTCGCAAGCTTGAGTGCACCGGAGCTGAGCCTTCTATTC
rev-xyr6-4	CCGGTGCACTCAAGCTTGCGAATTCGCCACTCGATCTCCCACCTTGAGGTC
fw-dga2-Cas9-inv	CGTAAGCAGTTCATGGATGACGGGTTTTAGAGCTAGAAATAGCA
rev-dga2-Cas9-inv	CCCGTCATCCATGAACTGCTTACGTCAACCTGCGCCGACCCGGA
fw-tgl4-Cas9-inv	CGTAGGCTCCTTGTCGAACACAGGTTTTAGAGCTAGAAATAGCA
rev-tgl4-Cas9-inv	CCTGTGTTCGACAAGGAGCCTACGTCAACCTGCGCCGACCCGGA
fw-oil1-Cas9-inv	CGTAGCAGTAACCTCGTGAACGGGTTTTAGAGCTAGAAATAGCA
rev-oil1-Cas9-	CCCGTTCACGAGGTTACTGCTACGTCAACCTGCGCCGACCCGGA

inv	
fw-dga1-knock_out-1	GTAGGGGTACCTCCCCGATC
rev-dga1-knock_out-2	ATGCAGTGGAGCCAGCAGAGGAATTGCGCAGCATAGCATG
fw-dga1-knock_out-3	CATGCTATGCTGCGCAATTCCTCTGCTGGCTCCACTGCAT
rev-dga1-knock_out-4	CTTCAACAGTATTTATTCCG
fw-dga2-knock_out-1	AAGAGCATCGTCCAGAACCC
rev-dga2-knock_out-2	AGATTACGTCAATGTCAATAGGCCAACTTCATGAGGCGCT
fw-dga2-knock_out-3	AGCGCCTCATGAAGTTGGCCTATTGACATTGACGTAATCT
rev-dga2-knock_out-4	GGGCTCTTTTGAGTAGCCT
fw-tgl4-knock_out-1	AGAAAGGCATTTGGACGAAC
rev-tgl4-knock_out-2	GGGTCCATCAAAGTACGACGTCGAATGAGCGAGATCTTTC
fw-tgl4-knock_out-3	GAAAGATCTCGCTCATTCGACGTCGTACTTTGATGGACCC
rev-tgl4-knock_out-4	ACCGACACAGACAGCACAGA

fw-oil1-knock_out-1	ATTTCTTTCCACACCACACA
rev-oil1-knock_out-2	ATGACCTTAGGTGCACATCAGTGCTGGTTGATTTCCTGAA
fw-oil1-knock_out-3	TTCAGGAAATCAACCAGCACTGATGTGCACCTAAGGTCAT
rev-oil1-knock_out-4	CGAACACCTCAATGGGCCAC

Table S3. A sequence of codon-optimized genes

atf1

ATGAACGAGATCGACGAGAAGAACCAGGCTCCTGTGCAGCAAGAGTGCCTGAAGGAAA
TGATCCAGAACGGACACGCCCGACGAATGGGCTCTGTCGAGGACCTGTACGTGGCCCTG
AACCGACAGAACCTGTACCGAAACTTCTGCACCTACGGCGAGCTGTCTGACTACTGCAC
CCGAGATCAGCTGACCCTGGCTCTGCGAGAGATCTGCCTGAAGAACCCTACTCTGCTGC
ATATCGTGCTGCCCACTCGATGGCCCAACCACGAGAACTACTACCGATCTTCTGAGTACT
ACTCTCGACCCCATCCTGTGCACGACTACATCTCCGTGCTGCAAGAGCTGAAGCTGTCTG
GCGTGGTGCTGAACGAGCAGCCCGAGTACTCTGCCGTGATGAAGCAGATCCTGGAAGAG
TTCAAGAACTCTAAGGGCTCTTACACCGCCAAGATCTTCAAGCTGACTACTACCCTGACC
ATTCCTTACTTCGGCCCCACTGGACCCTCTTGGCGACTGATCTGTCTGCCCGAGGAACAC
ACCGAGAAGTGGAAGAAGTTCATCTTCGTTTCTAACCACTGCATGTCTGACGGACGATCC
TCTATCCACTTCTTTCACGACCTGCGAGATGAGCTGAACAACATCAAGACCCCTCCAAAG
AAGCTGGACTACATTTTCAAGTACGAAGAGGACTACCAGCTGCTGCGAAAGCTGCCCGA
GCCTATCGAGAAGGTGATCGACTTCCGACCTCCTTACCTGTTCATCCCCAAGTCTCTGCT
GTCTGGATTCATCTACAACCACCTCCGATTCTCTTCGAAGGGCGTGTGCATGCGAATGGA

CGACGTGGAAAAGACCGACGACGTTGTGACCGAGATCATCAACATCTCTCCCACCGAGT

TCCAGGCCATCAAGGCCAACATTAAGTCTAACATCCAGGGCAAGTGTACCATCACTCCCT

TTCTGCACGTGTGCTGGTTCGTGTCTCTGCACAAGTGGGGCAAGTTCTTTAAGCCCCTGA

ACTTCGAGTGGCTGACCGACATCTTCATCCCCGCCGACTGCCGATCTCAGCTGCCTGACG

ACGACGAGATGCGACAGATGTACCGATACGGCGCCAACGTGGGCTTCATCGACTTCACC

CCTTGGATCTCTGAGTTCGACATGAACGACAACAAGGAAAACTTCTGGCCCCTGATCGA

GCACTACCACGAGGTGATTTCTGAGGCCCTGCGAAACAAGAAGCACCTCCACGGCCTGG

GCTTCAACATTCAGGGCTTCGTCCAGAAGTACGTCAACATTGACAAGGTGATGTGCGAC

CGAGCCATCGGCAAGCGACGAGGCGGCACCCTGCTGTCTAACGTGGGCCTGTTCAACCA

GCTCGAGGAACCCGACGCCAAGTACTCTATCTGCGACCTGGCCTTCGGCCAGTTCCAAG

GCTCTTGGCACCAGGCTTTCTCCCTGGGCGTGTGTTCTACCAACGTGAAGGGCATGAAC

ATCGTGGTGGCCTCTACCAAGAACGTGGTGGGCTCTCAAGAGTCTCTGGAAGAACTGTG

CTCTATCTACAAGGCCCTGCTGCTGGGCCCCTAA

atf2

ATGGAAGATATCGAGGGCTACGAGCCCCACATCACCCAAGAGCTGATCGACCGAGGACA

CGCCCGACGAATGGGCCACCTCGAGAACTACTTCGCCGTGCTGTCTCGACAGAAGATGT

ACTCTAACTTCACCGTGTACGCCGAGCTGAACAAGGGCGTGAACAAGCGACAGCTGATG

CTGGTGCTGAAGGTGCTGCTGCAGAAGTACTCTACCCTGGCTCACACCATCATTCCCAAG

CACTACCCTCACCACGAGGCCTACTACTCTTCCGAGGAATACCTGTCTAAGCCCTTTCCA

CAGCACGACTTCATCAAGGTGATCTCTCACCTCGAGTTCGACGACCTGATCATGAACAAC

CAGCCTGAGTACCGAGAGGTGATGGAAAAGATCTCTGAGCAGTTCAAGAAGGACGACTT

CAAGGTCACCAACCGACTGATCGAGCTGATCTCTCCCGTGATCATTCCCCTGGGCAACCC

CAAGCGACCCAACTGGCGACTGATCTGTCTGCCCGGCAAGGACACCGACGGCTTCGAG

ACTTGGAAGAACTTCGTGTACGTGACCAACCACTGCGGCTCTGACGGCGTGTCTGGATC

TAACTTCTTCAAGGACCTGGCTCTGCTGTTCTGCAAGATCGAGGAAAAGGGCTTCGACT

ACGACGAGGAATTCATCGAGGACCAGGTGATCATTGACTACGACCGAGACTACACTGAG

ATCTCTAAGCTGCCCAAGCCTATCACCGACCGAATCGACTACAAGCCCGCTCTGACCTCT

CTGCCCAAGTTCTTTCTGACCACCTTCATCTACGAGCACTGCAACTTCAAGACCTCTTCT

GAGTCTACCCTGACCGCTCGATACTCTCCCTCTTCTAACGCCAACGCCTCTTACAACTACC

TGCTGCACTTCTCTACCAAGCAGGTCGAGCAGATTCGAGCCCAGATCAAGAAGAACGTC

CACGACGGCTGCACTCTGACCCCTTTTATCCAGGCCTGCTTCCTGGTGGCCCTGTACCGA

CTGGACAAGCTGTTCACCAAGTCTCTGCTCGAGTACGGATTCGACGTGGCTATCCCCTCT

AACGCTCGACGATTCCTGCCTAACGATGAGGAACTGCGAGACTCTTACAAGTACGGCTCT

AACGTCGGCGGCTCTCACTACGCCTACCTGATCTCTTCGTTCGACATCCCCGAGGGCGAC

AACGACAAGTTCTGGTCCCTGGTCGAGTACTACTACGATCGATTCCTCGAGTCTTACGAC

AACGGCGACCACCTGATCGGCCTGGGCGTGCTGCAGCTGGACTTCATCGTCGAGAACAA

GAACATCGACTCCCTGCTGGCCAACTCTTACCTGCACCAGCAGCGAGGCGGAGCCATCA

TCTCTAACACCGGCCTGGTGTCTCAGGACACCACCAAGCCTTACTACGTGCGAGATCTGA

TTTTCTCTCAGTCTGCCGGCGCTCTGCGATTCGCCTTCGGCCTGAACGTGTGCTCTACCA

ACGTGAACGGCATGAACATGGACATGTCTGTGGTGCAGGGCACCCTGCGAGACAGAGG

CGAGTGGGAGTCTTTCTGTAAGCTGTTTTACCAGACTATCGGCGAGTTCGCCTCTCTGTA

A

<u>eat1 from *Kluyveromyces marxianus*</u>

ATGCTGCTGGCCTACACCGTGCGACCCTCTAACTGGTCTTTCACCCGACGAGCCTACTCT

GCCACCGCTCGAGCCTTCAACCAGCAGAAGGGACTGCTGCCCCTGCCTATCAAGGAAAC

CGTGGACATGGCCTACGACCTGCATCTGCCCGAGCGATCTGTGATCGGCAAGATGCCCTA

CCACTCTCCTGAGCCTATCATCTTCTACCACGGCCTGCTGGGCTCTAAGCGAAACTACCG

ACACGACTGCAAGAAGCTGGCTACCGCTCTGCAGACCCCTGTGTACACCGTCGACATCC

GAAACCACGGCTCCTCTGAGCACGCTCTGCCCTTCGACTACAACACCCTGGTGAACGAC

CTGGTGCACTTCGCCGAGACTCACTCTCTCGGCAAGGTGAACCTGGTGGGCTACTCTCT

GGGCGCCAAGGTGGCCATGCTGGCCTGCCTGAAGCACCCTGAGCGATTCTCTGCCGCCT

GCATCATCGACAACTCTCCCGAGGAACAGCCCCACATCAAGCCCCTGCTGACCGCTCTG

GTGAAGTCTTGCGTGAAGCTGCTGGACCACCACAACGTGCGAGCCGACGACAAGCTGT

GGCGACACAAGGCCTCTGAGGCCCTGAAGAAGTACATCCCCGACGCCGGCATCCGGTAC

TACCTGCTGTCTAACATCATCAACAACCCTCGAGTGGTCGAGTACCGATCTCCTGTGATC

AACTACGACGACGGCATGCTGCACTTCAAGAACCCCGTGCGACACATGATGGACTTCGT

GACCAAGGAAGTGGCCGCTTGGCCCACTCAAGAGCTGGAAGGCAAGCAGTTCCTGGGA

CCTGTGAACTTCATCAAGGCTACCCGATCTGACTTCATTAACCCCAAGTCTCTGCAGGCT

ATCAACCAGTACTTCCCCTACCACAACATCGACGAGATCAACGCTACCCACTTCATCCTG

AACGAGCGACCCCAAGAGTACCTGCGAGCCGTGACCGACTTCTTCAAGGTGACTCGATA

CCAGCTCGAGAAGAAGCGAGAGCAGGACCTGGCCAAGATCGACCAGCTGAACGCCTCC

GAGTCTCTGAAGTCTGCTCGAGACTAA

eat1 from *Wickerhamomyces anomalus*

ATGTTCTTCACCAAGGTGCTGAACAACCAGGTGGCCAACGGCCTGAAGCAGCTGCCCGT

GCACAAGCGAGTGCAGATGGCCTACGACCTGCACATCCCCAACAAGACTGTGAACCCCA

ACCTGAACATCCGATCTCACGAGCCCATCGTGTTCGTGCACGGCATCTTCGGCTCTAAGA

AGAACTACCGACACGACTGCCAAAAGATCGCCAACGTGACCCACACTCCTGTGTACACC

ATCGACCTGCGAAACCACGGCCAGTCTATGCACGCTCTGCCCTTCGACTACGAGACTCTG

GCCCAGGACGTGACCGACTTCTGCGAGGACCACGGACTGAAGAAGGTGAACCTGATCG

GCTACTCTCTGGGCGCCAAGATCTGCATGCTGACCATGCTGCAGAACCCCGACCTGGTGC

GATCTGGCGTGATCATCGACAACTCTCCCATCGAGCAGCCCCACATCGAGATCTTTCTGA

CCCAGTTCATCAAGTCTATGCTGCACGTGCTGAACTCTACCAAGATCCGAGCCGACGACA

AGGACTGGAAGTCTAAGGCCAACCAGGCCATGCGACGGTACATTCCCAACGGCGGCATC

CGAGACTACCTGCTGGCCAACCTGATCAACAAGGTGCCCAAGGGCTACAAGTCTCCCGT

GATCAACTACGACGACGGCTACATCCACTTTCAGAACCCTGTGCGACACATGACCGAGG

TGGCCGTGAAGAACGTGTCTGCTTGGCCCACCGAGCACGTGAAGGGCCTGAAGTTCGA

AGGCCAGGTGCGATTCCTGAAGGGCACCAAGTCTGCCTTCATCGACGAGAAGGGACTCG

AGGCCATCAAGGAATACTTCCCCAACTACTCCCTGTCTGAGCTGAACGCTACCCACTTCA

TCCTGAACGAGCGACCCCAAGAATACGTGAAGCTGATCTGCGACTTCATCAAGGTCAAC

CGATACAAGTCCCTGCAAGAGCACATCCGACACGTCGAGAACTTCTCGTCTGCCGAGCT

GGAAGCCCGACACAACGCCGAGCACGAGCGACAGATGGAAGAACTGCGACAGCTGACC

CAGACCACTCCTACCGCCGAGCAGGTCAAGACCATTGACAACCTGGCCTCTAAGGTGGA

CCTGTCTGCCACTGAGCGACAGCAGCAGCAGAACAAGGAAATCACCGTCTAA

<u>eat1 from *Wickerhamomyces ciferrii*</u>

ATGCACTTCACCCGAACTCTGTTCAACCAGGTGGCCTCTAAGGCCTCTCGACAGCTGCCC

GTGCAAAAGCGAGTGCAGATGGCCTACGACCTGCACATCCCCAACAAGACTGTGAACCC

CAACCTGAACATCCGATCTCACGAGCCCATCGTGTTCGTGCACGGCATCTTCGGCTCTAA

GAAGAACTACCGATTCGACTGCCAAAAGATCGCCAACGTGACCCACACTCCTGTGTACA

CCGTGGACCTGCGAAACCACGGCCAGTCTATCCACGCTCTGCCCTTCGACTACGAGACT

CTGGCCCAGGACGTGGCCGACTTCTGCGAGGACCACGGCCTGAAGAAGGTGAACCTGA

TCGGCTACTCTCTGGGCGCCAAGATCTGCATGCTGACCATGCTGCAGAACCCTGACCTGA

TCCGATCTGGCGTGATCATCGACAACTCTCCCATCGAGCAGCCCCACATCGAGATCTTTC

TGCAGATGTTCGTGAAGTCTATGATCCACGTGCTGAACTCTACCAAGATCGAGGCCAACG

ACTCTGACTGGAAGCGAAAGGCCGACGACGCCATGAAGCGGTACATCCCCGACGGCGG

CATCCGAAAGTACCTGCTGGCCAACCTGATCAACAAGGTGCCCAAGGGCTACAAGTCTC

CCGTGATTGACTACGACGACGGCTTCATCCACTTTCAGAACCCCGTGAAGCACATGACCG

122 AGGTGGCCGTGAAGAACGTGTCTGCTTGGCCCACCGAGAAGGTGGCCGGCAAGACCTT

123 CGAGGGCCCCATCCGATTCATCAAGGGCACCAAGTCTGCCTTCATTGACGACGCCGGAA

124 AGAAGGCCATTGCCGGCTACTTCCCCAACCACTCTATCTCTGAGATCAACGCTACCCACT

125 TCATCCTGAACGAGCGACCCCTCGAGTACGTGCGAGTGATCTGCGACTTCATCAAGACC

126 GAGCGATTCCGATCTCTGCAAGAGCACCTCCGAAACGTCGAGCACTTCTCGCCCTCTGA

127 GATTGAGGCTAAGCAGGCCGCCAAGCACGCCCAGCAGATCGAGGAACTGCGAAAGGTG

128 ACCTCTACCTCTGAGTCCTCTATTCCCCACTCTACCCAGTCCTCTGAGCAGGCCTTCACCG

129 AGAACATCGACCTGGCTCGACAAGAGCGAGAGCACCAGAAGTCTGTCTCTGCCTAA