

Supplementary Table S2 Primers used in this study

Primer Name	Sequence (5' - 3')	Description
<i>cat8</i> -F	atgccaggcatcctaccc	Amplification of the <i>cat8</i> gene
<i>cat8</i> -R	tcacgaggcttgaagaacag	
Cat8- <i>Sma</i> I-F	ctcgactctagaggatccccatgccaggcatcctacccatg	Amplification of the <i>cat8</i> gene
Cat8- <i>myc</i> ¹³ -R	cctcccatagagccaccgcccaggcttgaagaacagcagtg	
Cat8- <i>myc</i> ¹³ -F	attacgaattccccggggatctggtgcactctcagtacaatc	Amplification of the <i>myc</i> ¹³ and ADH1 terminator
<i>myc</i> ¹³ -R	cgataagcttgatatcgaattcacagatctatattacctgttatccc	
A-A-test-F	acgttaagtggatctcggtg	Verify that the <i>cat8-myc</i> ¹³ fragment is successfully recombined
A-A-test-R	catcttcccatccaagaacc	
<i>gpdA-cat8-myc</i> ¹³ -F	gctatgacatgattacgaattcgcagcggagagacggac	Amplification of the <i>cat8-myc</i> ¹³ cassette
<i>gpdA-cat8-myc</i> ¹³ -R	catcttctgtcgcaggaattcacagatctatattacctgttatccc	
cds-F	gcacgacaggtttcccgact	Verify that the <i>cat8-myc</i> ¹³ cassette is successfully recombined
cds-R	ctcaccgcctggacgactaa	
p426- <i>cat8</i> -F	gaaacagctatgacccgagg	Amplification of the upstream fragment of <i>cat8</i> sgRNA
SNpro- <i>cat8</i> -N-R	gtgcaatacgggattggcgggatcatttatcttctactcggag	
cr- <i>cat8</i> -N-F	cgccaatcccgtattgcacgttttagagctagaaatagcaag	Amplification of the downstream fragment of <i>cat8</i> sgRNA
p426- <i>cat8</i> -R	ttgtaaacgacggccagt	
p426-sgRNA-test-F	cacgacaggtttcccgactg	Verify that <i>cat8</i> sgRNA is successfully constructed
p426-N-test-R	gtgcaatacgggattggcg	
PC- <i>cat8</i> -up-F	cacaccgcatatgctggatcccacatgtttgctccacatgc	Construction of the upstream homology arm of <i>cat8</i> gene knockout
<i>cat8</i> -up- <i>hyg</i> -R	gataagcggcgttcaagttgg	
<i>cat8</i> -up- <i>hyg</i> -F	ccaactgaacgccgttatctcgcagagaagatgacattgaaggagc	Construction of the downstream homology arm of <i>cat8</i> gene knockout
PC- <i>cat8</i> -up-R	aggggttccgcgcaaagcttctcaggacattgttgagcc	
PC- <i>cat8</i> -down-F	cacaccgcatatgctggatccgtcaatacactacatggcgtg	

PC-down- <i>hyg</i> -R	aggggtccgcgcaaagcttaagaaggattaccttaacaagtg	
PC-down- <i>hyg</i> -F	ctgttttagaggaatccttctgtatagttggcgacccgg	
PC- <i>cat8</i> -down-R	aggggtccgcgcaaagcttcgagccattctatattcacagcc	
PCB004-F	cctttgagtgagctgataccg	
PCB004-R	agggtattgtctcatgagcg	Verify that the <i>cat8</i> knockout homology arm is successfully constructed
<i>cat8</i> -test-up-F	cgactcagattgctcgagagcg	
<i>cat8</i> -test-up-R	cgtcaggacattgttgagcc	Verify whether the split marker donor is successfully inserted into the knockout gene locus
<i>cat8</i> -test-down-F	gccgataacagcggtcattg	
<i>cat8</i> -test-down-R	ctacgaggcagcctttacgtg	
<i>actin</i> -F	gaagtgcgatgctgatgacaga	
<i>actin</i> -R	ggagcaaggcgggtgatt	<i>β-actin</i> relative expression level detection primer
qP- <i>cat8</i> -F-1	ctcgtattcttctcgggtgc	
qP- <i>cat8</i> -R-1	cggtgggttgatcattgc	<i>cat8</i> relative expression level detection primer
<i>icl1</i> -qF	gttggggagtatgaagatgctg	
<i>icl1</i> -qR	gaggaaggtagattgctgc	<i>icl1</i> relative expression level detection primer
<i>icl2</i> -qF	gactatgctgctcgtggtatgc	
<i>icl2</i> -qR	acacaccaccgaaaccatc	<i>icl2</i> relative expression level detection primer
<i>mls</i> -qF	tggctctaccgacagaaagatg	
<i>mls</i> -qR	atggcatcgtagaggttgagc	<i>mls</i> relative expression level detection primer
<i>adh</i> - <i>Sma</i> I-F	ctcgactctagaggatccccatgctggccactgagactatgaatatg	
<i>adh</i> - <i>EcoR</i> I-R	cgataagcttgatcgaattctcactcctgtttaaagtcgacaacaatcc	Amplification of the <i>adh</i> gene
<i>acs</i> - <i>Sma</i> I-F	cctcgactctagaggatccccatgctgacggagatgttaaaccgc	
<i>acs</i> - <i>EcoR</i> I-R	cgataagcttgatcgaattctactcttctgctgctgcacaacc	Amplification of the <i>acs</i> gene
pBAR- <i>pck</i> -F	ctcgactctagaggatccccatggttccaacagcgtcaacaag	
pBAR- <i>pck</i> -R	cgataagcttgatcgaattctcaagctgaggaccggcc	Amplification of the <i>pck</i> gene
<i>adh</i> - <i>acs</i> -F	ggcgggtgctctatgtctgacggagatgttaaaccg	Overlapping the <i>adh</i> and <i>acs</i>

<i>adh-acs</i> -R	gtcagacatagagccaccgccctcctgtftaaagtcgacaacaatccg	
<i>icl1</i> -F	atgcgtcgagcaaccattcag	
<i>icl1</i> -R	ctagaaactcctcctcgtgttacc	Amplification of the <i>icl1</i> gene
<i>icl2</i> -F	atgtcttcttctctcgcgacgc	
<i>icl2</i> -R	tcagtggftctcctcagccttg	Amplification of the <i>icl2</i> gene
<i>mls</i> -F	atggcacaacaagacc	
<i>mls</i> -R	ctacagcttgaggcaggag	Amplification of the <i>mls</i> gene
<i>eGFP</i> -F	tcgactctagaggatccccatggtgagcaaggcgag	Amplification of the <i>eGFP</i>
<i>icl1-eGFP</i> -R	tgaatggtgctcgcgcatgactgcagaattcgaagcttgag	Amplification of the <i>eGFP</i> fused with <i>icl1</i>
<i>icl2-eGFP</i> -R	tcgagagaagaagacatgactgcagaattcgaagcttgag	Amplification of the <i>eGFP</i> fused with <i>icl2</i>
<i>mls-eGFP</i> -R	tggtcttggtttgccatgactgcagaattcgaagcttgag	Amplification of the <i>eGFP</i> fused with <i>mls</i>
pBAR- <i>icl1</i> -R	gataagcttgatatcgaattctagaaactcctcgtgttacc	Amplification of <i>icl1</i> linking to P bargpe1
pBAR- <i>icl2</i> -R	gataagcttgatatcgaattcagtggtctcctcagccttg	Amplification of <i>icl2</i> linking to pBARGPE1
pBAR- <i>mls</i> -R	gataagcttgatatcgaattctacagctggaggcaggag	Amplification of the <i>mls</i> linking to pBARGPE1
<i>PgpdA</i> -test-F	cgcatccgcttacagaca	
<i>TrpC</i> -test-R	caggattgctgttatctgatgagt	Test the <i>icl/mls</i> cassette
pK2- <i>hyg-gpdA</i> -F	ctatgacatgattacgaattcggcatccgcttacagaca	
pK2- <i>hyg-trpC</i> -R	tcattctgtcgcgaattcaggtattgctgttatctgatgagt	Amplification of the <i>icl/mls</i> cassette
<i>gpdA-icl1</i> -F	tcgactctagaggatccccatgcgtcgagcaaccattcag	
<i>mls-icl1</i> -R	gagctcgagatctgagtcggagaaactcctcctcgtgttacc	Amplification of <i>icl1</i> for overlapping
Linker-F	tccggactcagatctcgagc	
pBAR- <i>mls</i> -R	cgataagcttgatatcgaattctacagcttgaggcaggag	Overlapping the <i>icl1</i> and <i>mls</i>
pK2- <i>hyg-gpdA</i> -F	ctatgacatgattacgaattcggcatccgcttacagaca	
pK2- <i>hyg-trpC</i> -R	tcattctgtcgcgaattcaggtattgctgttatctgatgagt	Amplification of the <i>icl-mls</i> cassette
<i>gpdA</i> -F	gctatgacatgattacgaattcgcgagagacggac	
pK2-R	catcttctgtcgcgaattcgcattgcagatgagctgtat	Amplification of the <i>adh /acs/adh-acs/cat8/pck</i> cassette

<i>Asp. PGI-F</i>	gggcagaaaagatagagaaaaggc	
<i>hyg-Asp. PGI-R</i>	gtgagttcaggcttttcatctgatgtttgtggaaaagtgg	Amplify the <i>Asp. PGI</i> gene
<i>Asp. FBA-F</i>	gcactgtcattgcaacctactg	
<i>hyg-Asp. FBA-R</i>	gtgagttcaggcttttcatctgatgtttgtggaaaagtgg	Amplify the <i>Asp. FBA</i> gene
<i>Asp. PGK-F</i>	gtgcgaagtaaaaccaaacagc	
<i>hyg-Asp. PGK-R</i>	gtgagttcaggcttttcatctgatgtttgtggaaaagtgg	Amplify the <i>Asp. PGK</i> gene
<i>Rt. PGI-F</i>	tggccgtctgccatttcacgtcg	
<i>hyg-Rt. PGI-R</i>	gtgagttcaggcttttcatctgatgtttgtggaaaagtgg	Amplify the <i>Rt. PGI</i> gene
<i>Rt. FBA-F</i>	ctctgctctcgcctcgtgtggcttg	
<i>hyg-Rt. FBA-R</i>	gtgagttcaggcttttcatctgatgtttgtggaaaagtgg	Amplify the <i>Rt. FBA</i> gene
<i>hyg-F</i>	atgaaaagcctgaactcaccgc	
<i>hyg-R</i>	ctatttctttgccctcggacg	Amplification of <i>hyg</i> for overlapping
<i>TrpC-hyg-R</i>	gataagcttgatcgaattctatttctttgccctcggacg	Construction of <i>promoter-hyg</i> expression cassette
<i>pBAR-Asp. PGI-F</i>	tcgactctagaggatccccgggcagaaaagatagagaaaaggc	Construction of <i>Asp. PGI-hyg</i> expression cassette
<i>pBAR-Asp. FBA-F</i>	tcgactctagaggatccccgcactgtcattgcaacctactg	Construction of <i>Asp. FBA-hyg</i> expression cassette
<i>pBAR-Asp. PGK-F</i>	tcgactctagaggatccccgtgcgaagtaaaaccaaacagc	Construction of <i>Asp. PGK-hyg</i> expression cassette
<i>pBAR-Rt. PGI-F</i>	tcgactctagaggatccccctgctcctcgtcctgctgtggcttg	Construction of <i>Rt. PGI-hyg</i> expression cassette
<i>pBAR-Rt. FBA-F</i>	tcgactctagaggatccccctgctcctcgtcctgctgtggcttg	Construction of <i>Rt. FBA-hyg</i> expression cassette
<i>pK2-Asp. PGI-F</i>	ctatgacatgattacgaattgggcagaaaagatagagaaaaggc	Construction of <i>pK2-Asp. PGI-hyg</i> plasmid
<i>pK2-Asp. FBA-F</i>	ctatgacatgattacgaattgcactgtcattgcaacctactg	Construction of <i>pK2-Asp. FBA-hyg</i> plasmid
<i>pK2-Asp. PGK-F</i>	ctatgacatgattacgaattgtgcgaagtaaaaccaaacagc	Construction of <i>pK2-Asp. PGK-hyg</i> plasmid
<i>pK2-Rt. PGI-F</i>	ctatgacatgattacgaattggccgtctgccatttcacgtc	Construction of <i>pK2-Rt. PGI-hyg</i> plasmid
<i>pK2-Rt. FBA-F</i>	ctatgacatgattacgaattctcctcctcgtcctgctgtggcttg	Construction of <i>pK2-Rt. FBA-hyg</i> plasmid
<i>pK2-TrpC-R</i>	agtggatccccgggaattcaggtattgctgttatctga	Construction of <i>pK2-promoter-hyg</i> plasmid
<i>qP-hyg-F</i>	tgtcctgcgggtaaatagc	
<i>qP-hyg-R</i>	tgtgaacggcgggagatg	<i>hyg</i> relative expression level detection primer

PC-pro-F	cggtatttcacaccgcatatggcatccgcttacagacaag	
<i>gpdA-CYC1</i> -R	gcgtgacataactaattacatgactcggtagcggcgaggacc	Amplify the <i>gpdA</i> promoter
Ter-F	gtcatgtaaftagttatgtcacgc	
PC- <i>CYC1</i> -R	aggggttccgcgcaaagcttgaccggccgcaaattaaag	Amplify the <i>CYC1</i> terminator
PC- <i>PGK-Nde I</i> -F	gcggtatttcacaccgcatatggcggaagtaaaacaaacagc	
PC- <i>PGK-Xba I</i> -R	agcccgggggacacctagatgtgactgaattggatgtgttagac	Amplify the <i>Asp.PGK</i> promoter
PC- <i>FBA-Nde I</i> -F	gcggtatttcacaccgcatatggcactgtcattgcaacctactg	
PC- <i>FBA-Xba I</i> -R	agcccgggggacacctagagtgtgattgtgtctgtgtgtgctg	Amplify the <i>Asp.FBA</i> promoter
PC- <i>PGI-Nde I</i> -F	gcggtatttcacaccgcatatggggcagaaaagatagagaaaaggc	
PC- <i>PGI-Xba I</i> -R	agcccgggggacacctagactgtatgtttgtgggaaaagtgg	Amplify the <i>Asp.PGI</i> promoter
PC- <i>Rt.FBA</i> -F	cacaccgcatatgctggatccctctgctctcgctcgctgtg	
<i>Rt.FBA</i> -R	tgtagctagttagttagaagtgaagtgg	Amplify the <i>Rt.FBA</i> promoter
PC- <i>Rt.PGI-Nde I</i> -F	gcggtatttcacaccgcatatggccgtctgccatttcacg	
PC- <i>Rt.PGI-Xba I</i> -R	agcccgggggacacctagaggctcgtagcgtggtgagtgaag	Amplify the <i>Rt.PGI</i> promoter
<i>cat8-Sma I</i> -F	ctcgactctagaggatccccatgccaggcatcctaccatg	
<i>cat8-EcoR I</i> -R	cgataagcttgatacgaattctcacgaggctgaagaacagcag	Amplification of the <i>cat8</i> gene
<i>PGK</i> -test-F	ctgctgctgtctccacacc	Verification of <i>PGK</i> promoter expression cassette
<i>FBA</i> -test-F	ctcataccacagcaaccagacaac	Verification of <i>FBA</i> promoter expression cassette
<i>PGI</i> -test-F	gtacacgcatcaccacccc	Verification of <i>PGI</i> promoter expression cassette
<i>Rt.FBA</i> -test-F	gctcgctcgtctctcgttg	Verification of <i>Rt.FBA</i> promoter expression cassette
<i>Rt.PGI</i> -test-F	gcttcactcaccagctacg	Verification of <i>Rt.PGI</i> promoter expression cassette
<i>CYC1</i> -test-R	catgcgtacacgcgtctgtac	Verification of the promoter library expression cassette reverse primer
<i>PGK</i> -pK2- <i>hyg</i> -F	gctatgacatgattacgaattcgtgcgaagtaaaacaaacagc	Amplified <i>PGK</i> promoter expression cassette
<i>FBA</i> -pK2- <i>hyg</i> -F	gctatgacatgattacgaattcgcactgtcattgcaacctactg	Amplified <i>FBA</i> promoter expression cassette
<i>PGI</i> -pK2- <i>hyg</i> -F	gctatgacatgattacgaattcggcgagaaaagatagagaaaaggc	Amplified <i>PGI</i> promoter expression cassette

<i>Rt.FBA</i> -pK2- <i>hyg</i> -F	gctatgacatgattacgaattcctctgctctcgctcgctgtg	Amplified <i>Rt.FBA</i> promoter expression cassette
<i>Rt.PGI</i> -pK2- <i>hyg</i> -F	gctatgacatgattacgaattctggccgtctgccattcatc	Amplified <i>Rt.PGI</i> promoter expression cassette
<i>CYC1</i> -pK2- <i>hyg</i> -R	catctctgtcgcgaattcgtaccggccgcaaattaaag	Amplified promoter library expression cassette reverse primer
