



21 **This document contains:**

22 **Figures S1-3**

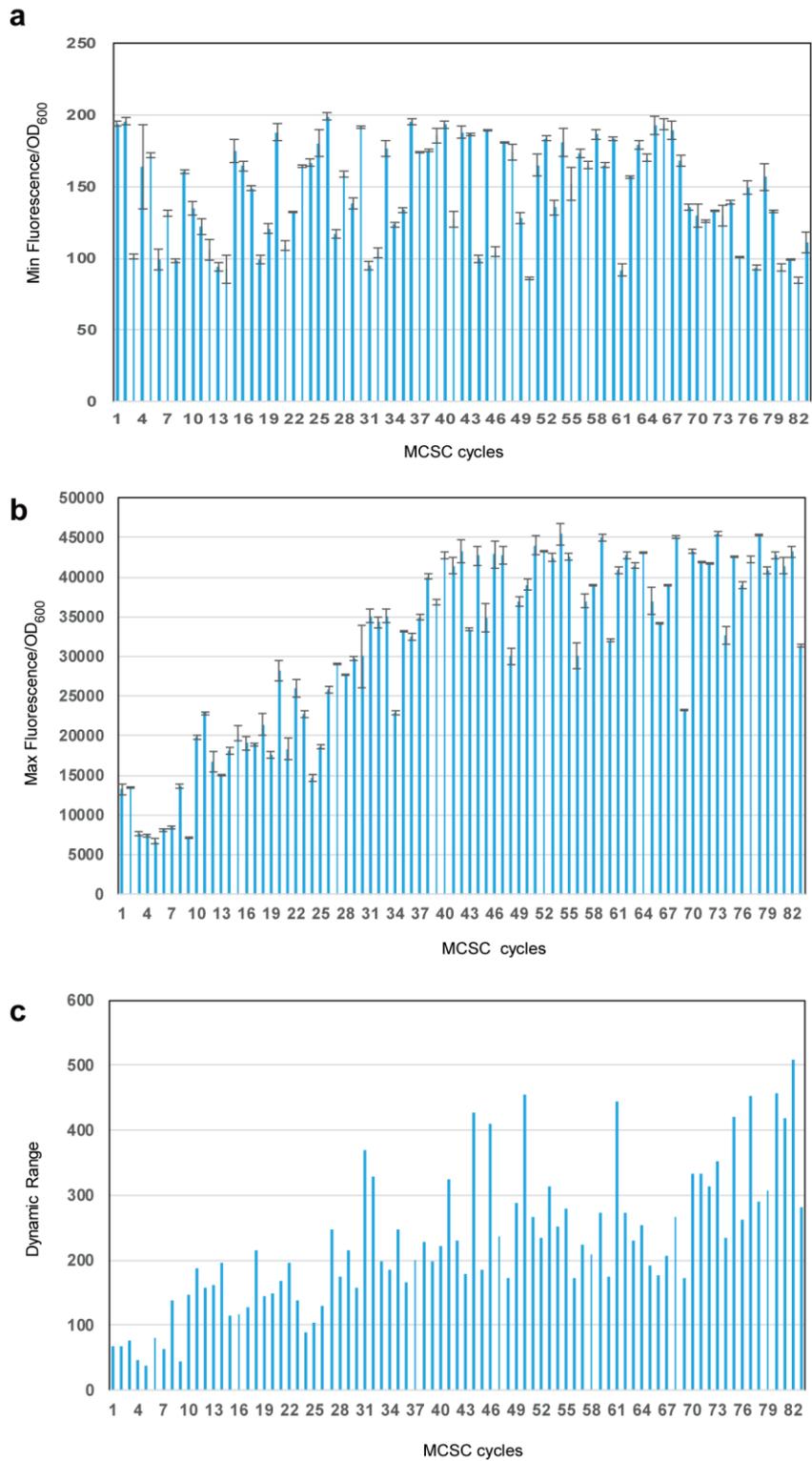
23 **Tables S1-2**

24 **Supplementary References**

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29 **Figure S1 The promoter library profiles of each round of MCSC engineering cycles.**

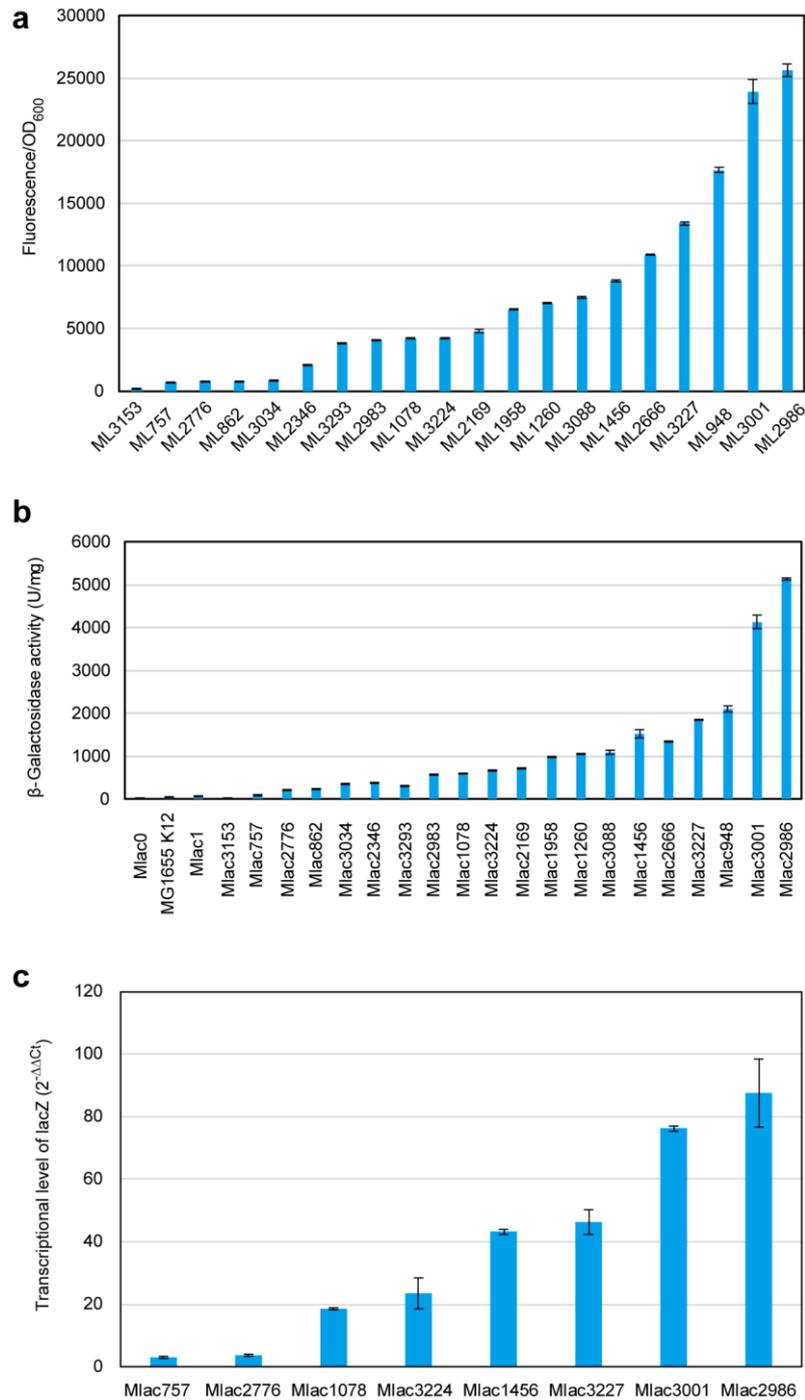
30 (a) The results of min fluorescence/OD<sub>600</sub> in every round of MCSC engineering. (b) The results of

31 max fluorescence/OD<sub>600</sub> in every round of MCSC engineering. (c) The results of dynamic range in

32 every round of MCSC engineering.

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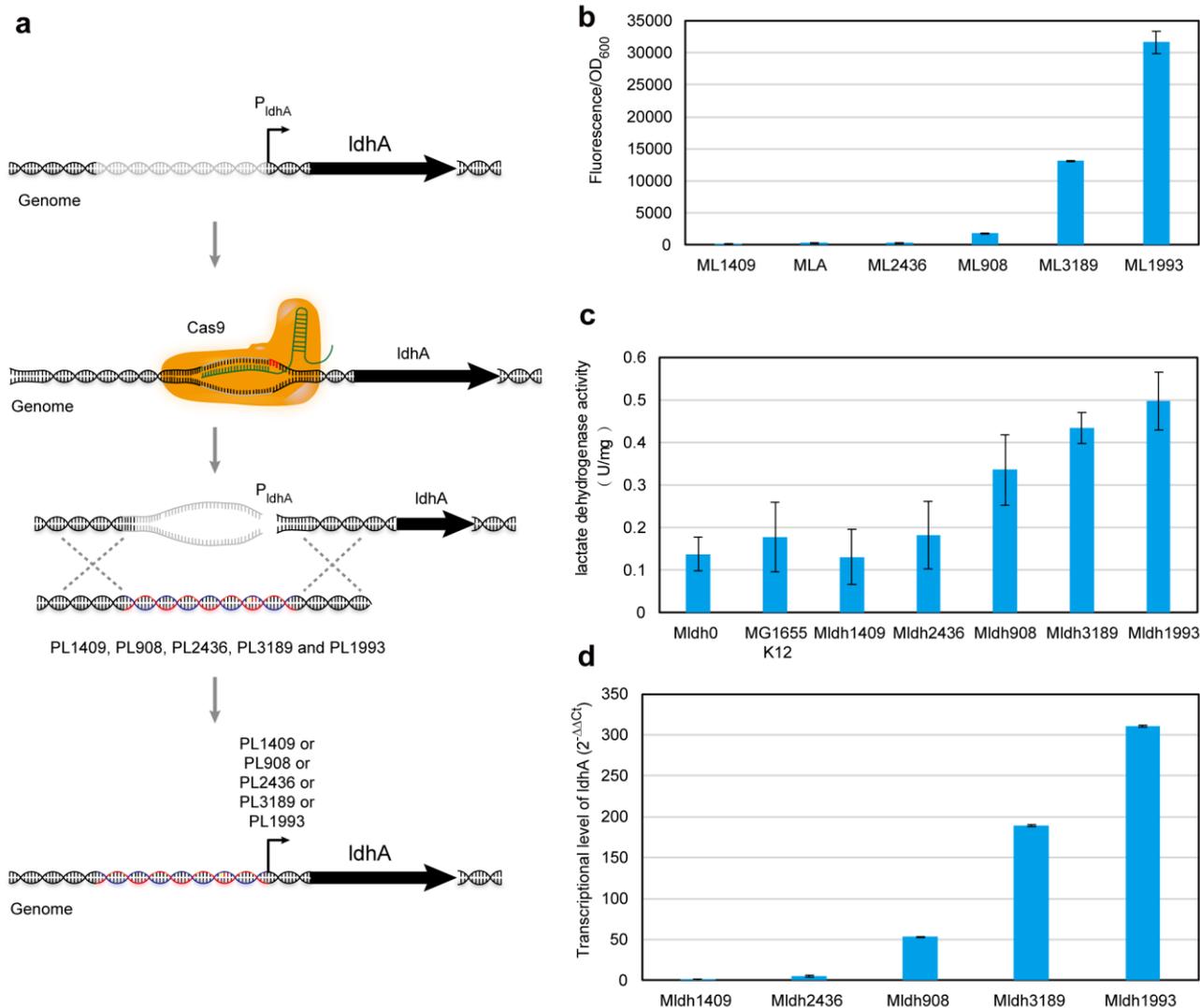
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36 **Figure S2 Comparing the fluorescence, LacZ activity, and transcriptional level of the**  
 37 **P<sub>trc</sub>-derived synthetic promoters.**

38 (a) Analysis of fluorescence intensities of the promoter library. ML0 (MG1655 carrying pL0-sfGFP  
 39 (pTrc99a harboring the sfGFP but no trc promoter)) was used as the negative control; MLN  
 40 (MG1655(DE3) carrying pLN-sfGFP (pL0-sfGFP harboring Trc promoter)) was used as the

41 positive control. (b) The activities of  $\beta$ -galactosidase under the control of different promoters were  
42 measured. Mlac0 was MG1655  $\Delta lacZ$ ; Mlac1 (MG1655  $\Delta lacZ$  harboring pLac1 (pTrc99a harboring  
43 the *lacZ*)) was used as the positive control. (c) Changes of activity of promoter candidates at the  
44 transcriptional level of *lacZ*. Level of changes of mRNA level( $2^{-\Delta\Delta C_t}$ ) of *lacZ* measured by real-time  
45 fluorescence quantitative PCR. All data and standard errors were derived from three independent  
46 biological replicates.

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49 **Figure S3 Comparing the fluorescence, LdhA activity, and transcriptional level of the**  
 50 **P<sub>trc</sub>-derived synthetic promoters.**

51 (a) The promoter replacement schematic on genome. (b) Analysis of fluorescence intensities of the  
 52 promoter library. ML0 (MG1655 carrying pL0-sfGFP (pTrc99a harboring the sfGFP but no trc  
 53 promoter)) was used as the negative control; MLA (MG1655 carrying pLA-sfGFP (pL0-sfGFP  
 54 harboring *E.coli* IdhA promoter)) was used as the positive control. (c) The activities of  
 55 β-galactosidase under the control of different promoters were measured. Mldh0 (MG1655 Δ  
 56 promoter IdhA) was used as the negative control; MG1655 K12 was used as the positive control. (d)  
 57 Changes of activity of promoter candidates at the transcriptional level of IdhA. Level of changes of

58 mRNA level( $2^{-\Delta\Delta C_t}$ ) of *ldhA* measured by real-time fluorescence quantitative PCR. All data and  
59 standard errors were derived from three independent biological replicates.

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61 **Table S1 Strains and plasmids used in this study**

<b>Strains and plasmids</b>	<b>Descriptions</b>	<b>Sources</b>
<b>Strains</b>		
JM109	For plasmid construction	Novagen
MG1655	For expressing genes	Novagen
MG1655(DE3)	For expressing genes	Novagen
ML0	MG1655 carrying pL0-sfGFP	This study
ML1	MG1655 carrying pL1-sfGFP	This study
ML2	MG1655 carrying pL2-sfGFP	This study
ML3	MG1655 carrying pL3-sfGFP	This study
ML4	MG1655 carrying pL4-sfGFP	This study
ML5	MG1655 carrying pL5-sfGFP	This study
ML6	MG1655 carrying pL6-sfGFP	This study
...	...	...
ML3665	MG1655 carrying pL3665-sfGFP	This study
MT7	MG1655(DE3) carrying pT7-sfGFP, Amp <sup>R</sup>	This study
MZ1	MG1655 carrying pZ1-sfGFP, Amp <sup>R</sup>	This study
MZ2	MG1655 carrying pZ2-sfGFP, Amp <sup>R</sup>	This study
MZ3	MG1655 carrying pZ3-sfGFP, Amp <sup>R</sup>	This study
MZ4	MG1655 carrying pZ4-sfGFP, Amp <sup>R</sup>	This study
...	...	...
MZ127	MG1655 carrying pZ127-sfGFP, Amp <sup>R</sup>	This study
Mlac0	MG1655 $\Delta$ lacZ	This study
Mlac1	MG1655 $\Delta$ lacZ harboring pLac1, Amp <sup>R</sup>	This study

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Mlac757	MG1655 $\Delta$ lacZ harboring pLac757, Amp <sup>R</sup>	This study
Mlac862	MG1655 $\Delta$ lacZ harboring pLac862, Amp <sup>R</sup>	This study
Mlac948	MG1655 $\Delta$ lacZ harboring pLac948, Amp <sup>R</sup>	This study
Mlac1073	MG1655 $\Delta$ lacZ harboring pLac1073, Amp <sup>R</sup>	This study
Mlac1078	MG1655 $\Delta$ lacZ harboring pLac1078, Amp <sup>R</sup>	This study
Mlac1260	MG1655 $\Delta$ lacZ harboring pLac1260, Amp <sup>R</sup>	This study
Mlac1456	MG1655 $\Delta$ lacZ harboring pLac1456, Amp <sup>R</sup>	This study
Mlac1958	MG1655 $\Delta$ lacZ harboring pLac1958, Amp <sup>R</sup>	This study
Mlac2169	MG1655 $\Delta$ lacZ harboring pLac2169, Amp <sup>R</sup>	This study
Mlac2346	MG1655 $\Delta$ lacZ harboring pLac2346, Amp <sup>R</sup>	This study
Mlac2666	MG1655 $\Delta$ lacZ harboring pLac2666, Amp <sup>R</sup>	This study
Mlac2776	MG1655 $\Delta$ lacZ harboring pLac2776, Amp <sup>R</sup>	This study
MLac2983	MG1655 $\Delta$ lacZ harboring pLac2983, Amp <sup>R</sup>	This study
Mlac2986	MG1655 $\Delta$ lacZ harboring pLac2986, Amp <sup>R</sup>	This study
Mlac3001	MG1655 $\Delta$ lacZ harboring pLac3001, Amp <sup>R</sup>	This study
Mlac3034	MG1655 $\Delta$ lacZ harboring pLac3034, Amp <sup>R</sup>	This study
Mlac3088	MG1655 $\Delta$ lacZ harboring pLac3088, Amp <sup>R</sup>	This study
Mlac3147	MG1655 $\Delta$ lacZ harboring pLac3147, Amp <sup>R</sup>	This study
Mlac3153	MG1655 $\Delta$ lacZ harboring pLac3153, Amp <sup>R</sup>	This study
Mlac3224	MG1655 $\Delta$ lacZ harboring pLac3224, Amp <sup>R</sup>	This study
Mlac3227	MG1655 $\Delta$ lacZ harboring pLac3227, Amp <sup>R</sup>	This study
Mlac3293	MG1655 $\Delta$ lacZ harboring pLac3293, Amp <sup>R</sup>	This study
MLA	MG1655 carrying pLA-sfGFP	This study
Mldh0	MG1655 $\Delta$ promoter ldhA	This study

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Mldh1409	MG1655 $\Delta$ promoter ldhA :: synthetic promoter 1409	This study
Mldh908	MG1655 $\Delta$ promoter ldhA :: synthetic promoter 908	This study
Mldh1845	MG1655 $\Delta$ promoter ldhA :: synthetic promoter 2436	This study
Mldh3189	MG1655 $\Delta$ promoter ldhA :: synthetic promoter 3189	This study
Mldh1993	MG1655 $\Delta$ promoter ldhA :: synthetic promoter 1993	This study
<b>Plasmids</b>		
pTrc99a	pBR322 ori, trc, Amp <sup>R</sup>	Novagen
pJKR-H	pUC ori, beta lactamase antibiotic resistance, High copy	(1)
pL0-sfGFP	pTrc99a harboring the sfGFP from pJKR-H, no trc promoter, Amp <sup>R</sup>	This study
pL1-sfGFP	pL0-sfGFP harboring synthetic promoter PL1(Trc), Amp <sup>R</sup>	This study
pL2-sfGFP	pL0-sfGFP harboring synthetic promoter PL2, Amp <sup>R</sup>	This study
pL3-sfGFP	pL0-sfGFP harboring synthetic promoter PL3, Amp <sup>R</sup>	This study
pL4-sfGFP	pL0-sfGFP harboring synthetic promoter PL4, Amp <sup>R</sup>	This study
pL5-sfGFP	pL0-sfGFP harboring synthetic promoter PL5, Amp <sup>R</sup>	This study
pL6-sfGFP	pL0-sfGFP harboring synthetic promoter PL6, Amp <sup>R</sup>	This study
...	...	....
pL3665-sfGFP	pL0-sfGFP harboring synthetic promoter PL3665, Amp <sup>R</sup>	This study
pT7-sfGFP	pL0-sfGFP harboring T7 promoter, Amp <sup>R</sup>	This study
pZ1-sfGFP	pL0-sfGFP harboring PZ1promoter, Amp <sup>R</sup>	This study
pZ2-sfGFP	pL0-sfGFP harboring PZ2 promoter, Amp <sup>R</sup>	This study
pZ3-sfGFP	pL0-sfGFP harboring PZ3 promoter, Amp <sup>R</sup>	This study
pZ4-sfGFP	pL0-sfGFP harboring PZ4 promoter, Amp <sup>R</sup>	This study
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pZ127-sfGFP	pL0-sfGFP harboring PZ127 promoter, Amp <sup>R</sup>	This study
pRSFDuet-1	RSF ori, lacI, T7 lac, Kan <sup>R</sup>	Novagen( 2)
placZ	pRSFDuet-1 harboring the <i>lacZ</i> gene <i>upstream</i> and downstream 500bp from <i>E. coli</i> , Kan <sup>R</sup>	This study
pLac1	pTrc99a harboring the <i>lacZ</i> from <i>E. coli</i> genome, Amp <sup>R</sup>	This study
pLac757	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 757, Amp <sup>R</sup>	This study
pLac862	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 862, Amp <sup>R</sup>	This study
pLac948	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 948, Amp <sup>R</sup>	This study
pLac1073	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 1073, Amp <sup>R</sup>	This study
pLac1078	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 1078, Amp <sup>R</sup>	This study
pLac1260	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 1260, Amp <sup>R</sup>	This study
pLac1456	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 1456, Amp <sup>R</sup>	This study
pLac1958	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 1958, Amp <sup>R</sup>	This study
pLac2169	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2169, Amp <sup>R</sup>	This study
pLac2346	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2346, Amp <sup>R</sup>	This study
pLac2666	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2666, Amp <sup>R</sup>	This study
pLac2776	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2776, Amp <sup>R</sup>	This study
pLac2983	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2983, Amp <sup>R</sup>	This study
pLac2986	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 2986, Amp <sup>R</sup>	This study
pLac3001	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 3001, Amp <sup>R</sup>	This study
pLac3034	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 3034, Amp <sup>R</sup>	This study
pLac3088	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 3088, Amp <sup>R</sup>	This study
pLac3147	pLac1- <i>lacZ</i> harboring mutant <i>trc</i> promoter 3147, Amp <sup>R</sup>	This study

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pLac3153	pLac1-lacZ harboring mutant trc promoter 3153, Amp <sup>R</sup>	This study
pLac3224	pLac1-lacZ harboring mutant trc promoter 3224, Amp <sup>R</sup>	This study
pLac3227	pLac1-lacZ harboring mutant trc promoter 3227, Amp <sup>R</sup>	This study
pLac3293	pLac1-lacZ harboring mutant trc promoter 3293, Amp <sup>R</sup>	This study
pLA-sfGFP	pL0-sfGFP harboring <i>E. coli</i> <i>ldhA</i> promoter, Amp <sup>R</sup>	This study
pldhA	pRSFDuet-1 harboring the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp from <i>E. coli</i> , Kan <sup>R</sup>	This study
pldh908	pldhA harboring mutant promoter 908 between the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp, Kan <sup>R</sup>	This study
pldh1409	pldhA harboring mutant promoter 1409 between the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp, Kan <sup>R</sup>	This study
pldh1993	pldhA harboring mutant promoter 1993 between the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp, Kan <sup>R</sup>	This study
pldh2436	pldhA harboring mutant promoter 2436 between the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp, Kan <sup>R</sup>	This study
pldh3189	pldhA harboring mutant promoter 3189 between the <i>ldhA</i> gene promoter <i>upstream</i> and downstream 500bp, Kan <sup>R</sup>	This study
pCas	paraB-gam-bet-exo, bla (kan <sup>R</sup> ), kanR-repA101 (ts), λ-Red, the sgRNA with a lacI <sup>q</sup> -P <sub>trc</sub> promoter guiding the pMB1 replication of pTarget	Addgene(3)
pTarget	the sgRNA sequence, a targeting N20 sequence, the pMB1 replicon, Spe <sup>R</sup>	Addgene(3)

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64 **Table S2 Primers used in this study**

<b>Primer</b>	<b>Sequence (5' to 3')</b>
pL0-sfGFP F	ATGGAATTCATGCGTATAGGTGAAGAACTGTTACCGGTGTTGTTC
pL0-sfGFP R	CAGCTCATTTTCAGAATATTTGCCAGAACCG
pL1-sfGFP F	CAGACCATGGAATTCATGCGTATAGGTGAAGAAC
pL1-sfGFP R	CAAACAGCCAAGCTTCTTTGTACAGTTCGTCCA
T7-F	ATATTCTGAAATGAGCTGTAATACGACTCACTATAGGGGAATTGT
T7-R	TATACGCATGAATTCCATGGTATATCTCCTTATTAAAGTTAAACAAAATT
er-Trc F	ACATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTA ATCATCCGGC
er-Trc R	ACCGGTGAACAGTTCTTCACCTATACGCATGAATTCCATGGTCTGTTTCC TGTGTGAAA
pT7-sfGFP F	TAATACGACTCACTATAATGGAATTCATGCGTATAGGTGAAGAACTGTT CACCGGTG
pT7-sfGFP R	CAGCTCATTTTCAGAATATTTGCCAGAACCG
Miseq F	CCTACACGACGCTCTTCCGATCTCGCCCACCGGCAGCCATCGGAAGCTG TGGTATGGCTGT
Miseq R	GGAGTTCCTTGGCACCCGAGAATTCCACTAGTCGGCCACGGAACCGGCA GTTTACCGGTGG
veri-pTrc F	AGGCAGCCATCGGAAGCTGTGGTA
veri-pTrc R	GAGACCCACACTACCATCGGCG
veri-T0 F	GAAATGAGCTGATGGAATTCATGCGTATAGG
veri-lacZ	GCCCGAGTTTGTGAGAAAGCCCGTCTTCAACGTTGTGACGGTAATACGA
Rveri	CTCACTATAGAAATGAGCTGATGGAATTCATGCGTATAGG

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Rveri-T7

Fveri-T0 F

Seq2FplacZ  
down FplacZ  
up Rveri

Rveri-T0

Fveri-ssrS F

Seq2RplacZ  
down RplacZ  
down FplacZ  
up Fveri-tet

Fveri-T0 F

q16s

Fveri-lacZ

FplacZ down

RplacZ up

Rveri-T7

Fveri-tet F

q16s

Rveri-lacZ

Rveri-lacZ

FplacZ down

Fveri

AGGCAGCCATCGGAAGCTGTGGTAGAATTGTGAGCGGATAACAATTTCA  
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GAAATTGTTATCCGCTCCCGTCTTCAACGTTGTGACGGGAAATGAGCTG  
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CGTATAGG  
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GCTGTTTCCTGTGTGAAATTGTTATCCGCTCTAATACGACTCACTATAAG  
TGATAGAGATTGACATCCC  
TAACCCAACATTTCACAACAGCCCGAGTTTGTCAGAAAGCACTTTATGC  
TTCCGGCTCGTGAATTGTGAGCGGATAACAATTTACACAGGAAACAGC  
TTAATAACCGGGCAGGCCATGCCGTCTTCAACGTTGTGACGGTAATACG  
ACTCACTATA

Rveri-T7 F

qlacZ TCAACATCAGCCGCTACAGTCAACGGCAGCCATCGGAAGCTGTGGTATG

FSeqFveri-la GCTGTGCCCCGAGTTTGTTCAGAAAGCACTTTCTGTTCGACTTAAGCATTAT

cZ RplacZ GCGGCCGCAAGCTTCCAACACAGCCAAACATCCGCAGCCATCACCATCA

down RplacZ TCACCACAGCCAGGATCCGAATTCGGCATCGTTCCCACTGCGATCCGTC

up Fveri R TTCAACGTTGTGACGG

qlacZ

RSeqRSeqFv ATTCAGCCATGTGCCTTCTTCCGTCGGCCACGGAACCGGCAGTTTACCG

eri-lacZ GTGGGGCAGCCATCGGAAGCTGTGGTATGGCTGTACTIONTATGCTTCCGG

FplacZ up CTCGTAGCTGTTTCTGTGTGAAATTGTTATCCGCTCCAGCCATCACCAT

RplacZ up F CATCACCACAGCCAGGATCCGAATTCGGCATCGTTCCCACTGCGAT

qsfGFP

FSeq2FSeqR GGTGAAGGTGACGCTACCAACGAGGCAGCCATCGGAAGCTGTGGTATC

veri-lacZ GGCCACGGAACCGGCAGTTTACCGGTGGGCCCCGAGTTTGTTCAGAAAGCG

RplacZ down AATTGTGAGCGGATAACAATTTACACACAGGAAACAGCTTAATAACCGGG

FplacZ up R CAGGCCATGAGCTGTTTCTGTGTGAAATTGTTATCCGCTC

qsfGFP

RSeq2RSeq2 GCGAAGCACTGAACACCGTAGGCCGGGTAACGAGCGAAGCACTGAAGGC

FSeqFplacZ AGCCATCGGAAGCTGTGGTAGGCAGCCATCGGAAGCTGTGGTATGGCTG

down RplacZ TACTTTCTGTTCGACTTAAGCATTATGCGGCCGCAAGCTTCCAACACAGC

down F CAAACATCCGGAATTGTGAGCGGATAACAATTTACACACAGGAAACAGCT

pLA Fq16s TAATAACCGGGCAGGCCATG

FSeq2RSeqR GAAGGTTGCGCCTACACTAATCTTGACATCCACAGAACTTCGGGTAACG

veri-lacZ AGCGAAGCACTGATCGGCCACGGAACCGGCAGTTTACCGGTGGACTTTA

TGCTTCCGGCTCGTACTTTCTGTTCGACTTAAGCATTATGCGGCCGCAAG

FplacZ down CTTCCAACACAGCCAAACATCCG

R

pLA Rq16s

Rq16s AAGACTTTCTCCAGTGATGTTGTAACCCAACATTCACAACATCTTGACA

FSeq2Fveri-l TCCACAGAACTTAGGCAGCCATCGGAAGCTGTGGTAGCCCGAGTTTGTC

acZ AGAAAGCACTTTATGCTTCCGGCTCGT

Rveri-lacZ F

pLA-sfGFP

FqlacZ ACATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGGAAGGTTGCG

Fq16s CCTACACTAATCAACATCAGCCGCTACAGTCAACTAACCCAACATTTCA

RSeq2RSeqF CAACACGGGTAACGAGCGAAGCACTGAGGCAGCCATCGGAAGCTGTGG

veri-lacZ R TATGGCTGTGCCCCGAGTTTGTCAGAAAGC

pLA-sfGFP

RqlacZ AACACCGGTGAACAGTTCTTCACCTATACGCATGAATTCAAGACTTTCTC

RqlacZ CAGTGATGTATTCAGCCATGTGCCTTCTTCCGTCAACATCAGCCGCTACA

Fq16s GTCAACTCTTGACATCCACAGAACTTTCGGCCACGGAACCGGCAGTTTA

FSeqRSeqF CCGGTGGGGCAGCCATCGGAAGCTGTGGTATGGCTGT

pldhA up

FqsfGFP CAGCCATCACCATCATCACCACAGCCAGGATCCGAATTCCCCTGCCATT

FqlacZ CCTGCCAGGGGGTGAAGGTGACGCTACCAACGATTCAGCCATGTGCCTT

Rq16s CTTCCGTAAACCAACATTCACAACAAGGCAGCCATCGGAAGCTGTGGT

RSeq2FSeqR ATCGGCCACGGAACCGGCAGTTTACCGGTGG

pldhA up

RqsfGFP CTTGTCTGACTGTTTTGTGCTATAAACGGCGAGTTTCATAACTGAACGGT

TAAACATGCGCGAAGCACTGAACACCGTAGGGGTGAAGGTGACGCTAC

RqsfGFP CAACGTCAACATCAGCCGCTACAGTCAACCGGGTAACGAGCGAAGCAC  
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F  
pldhA down  
FpLA  
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FqsfGFP  
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RqlacZ  
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Rq16s  
FSeq2R  
pldhA down  
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RpLA RpLA  
CAGAATGCGAAGACTTTCTCCAGTGATGTTGGAAGGTTGCGCCTACACT  
FqsfGFP  
AAGGTGAAGGTGACGCTACCAACGTAACCCAACATTTCAACAACATCTTG  
Fq16s Rq16s  
ACATCCACAGAACTT  
F  
pldh908  
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FpLA-sfGFP  
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FpLA  
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RqsfGFP  
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RqlacZ  
TTTCAACA  
Fq16s R  
pldh908  
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RpLA-sfGFP  
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RpLA-sfGFP  
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 FqlacZ ATTCAGCCATGTGCCTTCTTCCGTCAACATCAGCCGCTACAGTCAAC  
 RqlacZ F  
 pldh1409  
 FpldhA up TGTTGTTCCGATCCTGGTTGAACTGGACGGTGACGTATGAAACTCGCCG  
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 FpLA-sfGFP CCCTGCCATTCTGCCAGGGAACACCGGTGAACAGTTCTTCACCTATAC  
 RpLA GCATGAATTCAAGACTTTCTCCAGTGATGTAAGACTTTCTCCAGTGATGT  
 RqsfGFP TGGGTGAAGGTGACGCTACCAACGATTCAGCCATGTGCCTTCTTCCG  
 FqlacZ R  
 pldh1409 CCGGTGAACGGTTCTTCACCTATACGCATGAATTCCATAACTGAACGGT  
 RpldhA up TAAACATGCCCTTGTCGTA CTGTTTTGTGCTATAAACGGCGAGTTTCATA  
 RpldhA up ACTGAACGGTTAAACATGCCAGCCATCACCATCATCACCACAGCCAGGA  
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 FqsfGFP AATATTCTGAAATGAGCTGGAAGGTTGCGCCTACACTAAGCGAAGCACT  
 RqsfGFP F GAACACCGTAGGGGTGAAGGTGACGCTACCAACG  
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 FqsfGFP R  
 pldh1993 AATTCCACACATTATACGAGCCGGATGATTAATTGTCAAAACTGAACGG  
 RpldhA TTAAACATGCACTTTCTGTTTCGACTTAAGCATTATGCGGCCGCAAGCTTC

down CAAAACCTTTCAGAATGCGATGAAACTCGCCGTTTATAGCACCAGCCAT  
RpldhA CACCATCATCACCACAGCCAGGATCCGAATTCCCCTGCCATTCTGCCA  
down GGAAGACTTTCTCCAGTGATGTTGGAAGGTTGCGCCTACTACTAA  
FpldhA up  
FpLA RpLA  
F  
pldh2436 TGTGAGCGGATAACAATTTACACACAGGAAACAGACCATGAAACTCGCC  
Fpldh908 GTTTATAGCACTGTGGGCGGATAACACTTTACACATGAAACAGACCAT  
FpldhA GAAACTCGCCGTTTATAGCACACTTTCTGTTCGACTTAAGCATTATGCGG  
down CCGCAAGCTTCCAAAACCTTTCAGAATGCGCTTGTCGTACTGTTTTGTGC  
RpldhA up TATAAACGGCGAGTTTCATAACTGAACGGTTAAACATGCACATCATAAC  
RpLA-sfGFP GGTCTGGCAAATATTCTGAAATGAGCTGGAAGGTTGCGCCTACTACTAA  
FpLA R AAGACTTTCTCCAGTGATGTTG  
pldh2436  
Rpldh908 ATTCCACACATTATACGAGCCGAATGATTAATTGTCAAAACTGAACGGT  
Rpldh908 TAAACATGCCATTCCACACACTATACGAGCCGGATGATTAATTGTCAA  
FpldhA ACTGAACGGTTAAACATGCCTGTGGGCGGATAACACTTTACACATGAA  
down ACAGACCATGAAACTCGCCGTTTATAGCACATGAAACTCGCCGTTTATA  
FpLA-sfGFP GCACAACACCGGTGAACAGTTCTTCACCTATACGCATGAATTCAAGACT  
RpLA-sfGFP TTCTCCAGTGATGTACATCATAACGGTTCTGGCAAATATTCTGAAATGA  
F GCTGGAAGGTTGCGCCTACTACTAA  
pldh3189 TGTAAGCGGATAACAATTTACACACAGGAAACAGACCATGAAACTCGCC  
Fpldh1409 GTTTATAGCACTGTTGTTCCGATCCTGGTTGAACTGGACGGTGACGTATG  
Fpldh908 AAACTCGCCGTTTATAGCACATTCCACACACTATACGAGCCGGATGATT

RpldhA AATTGTCAAACACTGAACGGTTAAACATGCCACTTTCTGTTCGACTTAAG  
down CATTATGCGGCCGCAAGCTTCCAAAACCTTTCAGAATGCGCAGCCATCA  
RpldhA up CCATCATCACCACAGCCAGGATCCGAATTCCCCTGCCATTCCCTGCCAGG  
FpLA-sfGFP GAACACCGGTGAACAGTTCTTCACCTATACGCATGAATTCAAGACTTTC  
R TCCAGTGATGT  
ATTCACACATTATACGAGCCGGATGATTAATTGTCAAACACTGAACGGTT  
pldh3189 AAACATGCCACCGGTGAACGGTTCTTCACCTATACGCATGAATTCCATA  
Rpldh1409 ACTGAACGGTTAAACATGCCTGTTGTTCCGATCCTGGTTGAACTGGACG  
Rpldh1409 GTGACGTATGAAACTCGCCGTTTATAGCACTGTGGGCGGATAAACTTT  
Fpldh908 CACACATGAAACAGACCATGAAACTCGCCGTTTATAGCACCTTGTCGTA  
FpldhA up CTGTTTTGTGCTATAAACGGCGAGTTTCATAACTGAACGGTTAAACATG  
RpldhA up F CCAGCCATCACCATCATCACCACAGCCAGGATCCGAATTCCCCTGCCAT  
TCCTGCCAGGG  
veri-ldhA  
Fpldh1993 CGCCATAGCTTTCAATTAATTTGGTGAGTGGATAACAATTTACACACAG  
Fpldh1409 GAAACAGACGATGAAACTCGCCGTTTATAGCACACCGGTGAACGGTTCT  
Rpldh908 TCACCTATACGCATGAATTCCATAACTGAACGGTTAAACATGCCATTCC  
RpldhA ACACACTATACGAGCCGGATGATTAATTGTCAAACACTGAACGGTTAAAC  
down ATGCCATGAAACTCGCCGTTTATAGCACCTTGTCGTA CTGTTTTGTGCTA  
FpldhA up R TAAACGGCGAGTTTCATAACTGAACGGTTAAACATGC  
veri-ldhA CTTTAATAAGGAGATATACCATGAATTCCACACATTATACGAGCCGGAT  
Fpldh1993 GATTAATTGTCAAACACTGAACGGTTAAACATGCGTGAGTGGATAACAAT  
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Fpldh1409 TCCGATCCTGGTTGAACTGGACGGTGACGTATGAAACTCGCCGTTTATA

FpldhA GCACACTTTCTGTTGCGACTTAAGCATTATGCGGCCGCAAGCTTCCAAAA  
 down CCTTTCAGAATGCGATGAAACTCGCCGTTTATAGCAC  
 RpldhA  
 down F  
 veri-ldh908 GTGTGTGGAATTGTGGGCGGATAACTGTGAGCGGATAACAATTTACACAC  
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 down R AGCTTCCAAAACCTTTCAGAATGCG  
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 Fpldh908 GCACAATTCCACACACTATACGAGCCGGATGATTAATTGTCAAAACTGA  
 Rpldh908 F ACGGTAAACATGCCTGTGGGCGGATAAACTTTACACATGAAACAGA  
 CCATGAAACTCGCCGTTTATAGCAC  
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 Fpldh3189 CAGGAAACAGACCATGAAACTCGCCGTTTATAGCACATTCCACACATTA  
 Fpldh2436 TACGAGCCGAATGATTAATTGTCAAAACTGAACGGTTAAACATGCCAAT  
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 Rpldh1409 AACATGCTGTTGTTCCGATCCTGGTTGAACTGGACGGTGACGTATGAAA  
 Fpldh908 R CTCGCCGTTTATAGCACATTCCACACACTATACGAGCCGGATGATTAATT

GTCAAAACTGAACGGTTAAACATGCC

veri-ldh2436 TGTGTGGAATTGTGAGCGGATAACATTCACACATTATACGAGCCGGATG  
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Rpldh3189 ATTTACACACAGGAAACAGACCATGAAACTCGCCGTTTATAGCACTGTGA  
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Fpldh1409 AGCACCCGGTGAACGGTTCTTCACCTATACGCATGAATTCCATAACTGA  
Rpldh1409 F ACGGTAAACATGCCTGTTGTTCCGATCCTGGTTGAACTGGACGGTGAC  
GTATGAAACTCGCCGTTTATAGCAC  
veri-ldh3189 ATGTGTGAATTGTAAGCGGATAACCGCCATAGCTTTCAATTAATTTGA  
Fveri-ldhA TTCACACATTATACGAGCCGGATGATTAATTGTCAAAACTGAACGGTTA  
Fpldh3189 AACATGCCAATTCCACACATTATACGAGCCGAATGATTAATTGTCAAAA  
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Fpldh1409 R TATACGCATGAATTCCATAACTGAACGGTTAAACATGCC  
veri-ldh CTTCTTATACTTAACTAATATACTAAGACTTTAATAAGGAGATATACCAT  
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Rpldh1993 F AGCACA  
qldhA GCGTTCGATCCGTATCCAAGTGGTGTGTGGAATTGTGGGCGGATAACC  
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Fveri-ldhA TAATTGTCAAAACTGAACGGTTAAACATGCCATGTGAGCGGATAACAAT  
Fpldh3189 TTCACACAGGAAACAGACCATGAAACTCGCCGTTTATAGCACAATTCCA

Rpldh2436 CACATTATACGAGCCGGATGATTAATTGTCAAAACTGAACGGTTAAACA  
 Fpldh1993 R TGC  
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 9 TGTGGAATTGTGGGCGGATAACCGCCATAGCTTTCAATTAATTTGATTC  
 Fveri-ldh908 CACACATTATACGAGCCGAATGATTAATTGTCAAAACTGAACGGTTAAA  
 Fveri-ldhA CATGCCTGTGAGCGGATAACAATTTACACACAGGAAACAGACCATGAAA  
 Fpldh2436 CTCGCCGTTTATAGCAC  
 Rpldh2436 F  
 sgRNA1(ldh  
 A)veri-ldh19 TAAATGTGATTCAACATCAC  
 93 TGGAATGTGTGGAATTGTGAGTGGATAACCCGTTCCACCGGTGTTGTT  
 CCGATCCCTTTAATAAGGAGATATACCATGTGTAAGCGGATAACAATTT  
 Fveri-ldh140 CACACAGGAAACAGACCATGAAACTCGCCGTTTATAGCACATTCCACAC  
 9 Fveri-ldhA ATTATACGAGCCGAATGATTAATTGTCAAAACTGAACGGTTAAACATGC  
 Fpldh3189 C  
 Fpldh2436 R  
 sgRNA2(ldh  
 A)veri-ldh24 TCAAATTTAATTGAAAGCTA  
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 3 GCCGGATGATTAATTGTCAAAACTGAACGGTTAAACATGCCATGTAAGC  
 Fveri-ldh908 GGATAACAATTTACACACAGGAAACAGACCATGAAACTCGCCGTTTATAG  
 Fpldh3189 CAC  
 Rpldh3189 F

veri-ldh3189

Fveri-ldh243 ATGTGTGAATTGTAAGCGGATAACTGTGTGGAATTGTGAGCGGATAACC

6 CGTTCACCGGTGTTGTTCCGATCCCGCCATAGCTTTCAATTAAATTTGAT

Fveri-ldh140 TCACACATTATACGAGCCGGATGATTAATTGTCAAAACTGAACGGTTAA

9 Fveri-ldhA ACATGCCA

Fpldh3189 R

veri-ldh

Rveri-ldh318

9 CTTCTTATACTTAACTAATACTAAGAATGTGTGAATTGTAAGCGGATA

Fveri-ldh199

3 Fveri-ldhA

ACAATGTGTGGAATTGTGAGTGGATAACCTTTAATAAGGAGATATACCA

TGCGCCATAGCTTTCAATTAAATTTG

Fveri-ldhA F

qldhA

Fveri-ldh

Rveri-ldh243

6

Fveri-ldh908

Fveri-ldhA F

qldhA

RqldhA

Fveri-ldh318

9

Fveri-ldh140

9

GGCGTTCGATCCGTATCCAAGTGCTTCTTATACTTAACTAATACTAAG

ATGTGTGGAATTGTGAGCGGATAACGTGTGTGGAATTGTGGGCGGATAA

CCTTTAATAAGGAGATATACCATG

AGGCGGCTTCGTTCAACAGATGGGCGTTCGATCCGTATCCAAGTGATGT

GTGAATTGTAAGCGGATAACCCGTTACCGGTGTTGTTCCGATCCGTGT

GTGGAATTGTGGGCGGATAAC

Fveri-ldh908  
F  
sgRNA1(ldh  
A)qldhA TAAATGTGATTCAACATCAC  
Rveri-ldh TGGAGGCGGCTTCGTTCAACAGATGCTTCTTATACTTAACTAATATA  
Rveri-ldh199 CTAAGAAATGTGTGGAATTGTGAGTGGATAACCCGTTACCCGGTGTGTG  
3 TCCGATCC  
Fveri-ldh140  
9 F  
sgRNA2(ldh  
A)sgRNA1(l  
dhA)qldhA TCAAATTTAATTGAAAGCTA TGGTAAATGTGATTCAACATCAC  
Fveri-ldh243 TGGGGCGTTCGATCCGTATCCAAGTGTGTGTGGAATTGTGAGCGGAT  
6 AACAAATGTGTGGAATTGTGAGTGGATAAC  
Fveri-ldh199  
3 F  
sgRNA2(ldh  
A)qldhA TCAAATTTAATTGAAAGCTA  
Rveri-ldh318 TGGAGGCGGCTTCGTTCAACAGATGATGTGTGAATTGTAAGCGGAT  
9 AACTGTGTGGAATTGTGAGCGGATAAC  
Fveri-ldh243  
6 F  
sgRNA1(ldh TAAATGTGATTCAACATCAC  
A)veri-ldh TGGCTTCTTATACTTAACTAATATACTAAGAATGTGTGAATTGTAAG

Rveri-ldh318 CGGATAAC

9 F

sgRNA2(ldh TCAAATTTAATTGAAAGCTA

A)ql dhA TGGGGCGTTCGATCCGTATCCAAGTGCTTCTTATACTTAACTAATAT

Fveri-ldh R ACTAAGA

ql dhA

AGGCGGCTTCGTTCAACAGATGGGGCGTTCGATCCGTATCCAAGTG

Rql dhA F

sgRNA1(ldh

TAAATGTGATTCAACATCAC TGGAGGCGGCTTCGTTCAACAGATG

A)ql dhA R

sgRNA2(ldh

A)sgRNA1(1 TCAAATTTAATTGAAAGCTA TGGTAAATGTGATTCAACATCACTGG

dhA)

sgRNA2(ldh

TCAAATTTAATTGAAAGCTA TGG

A)

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