

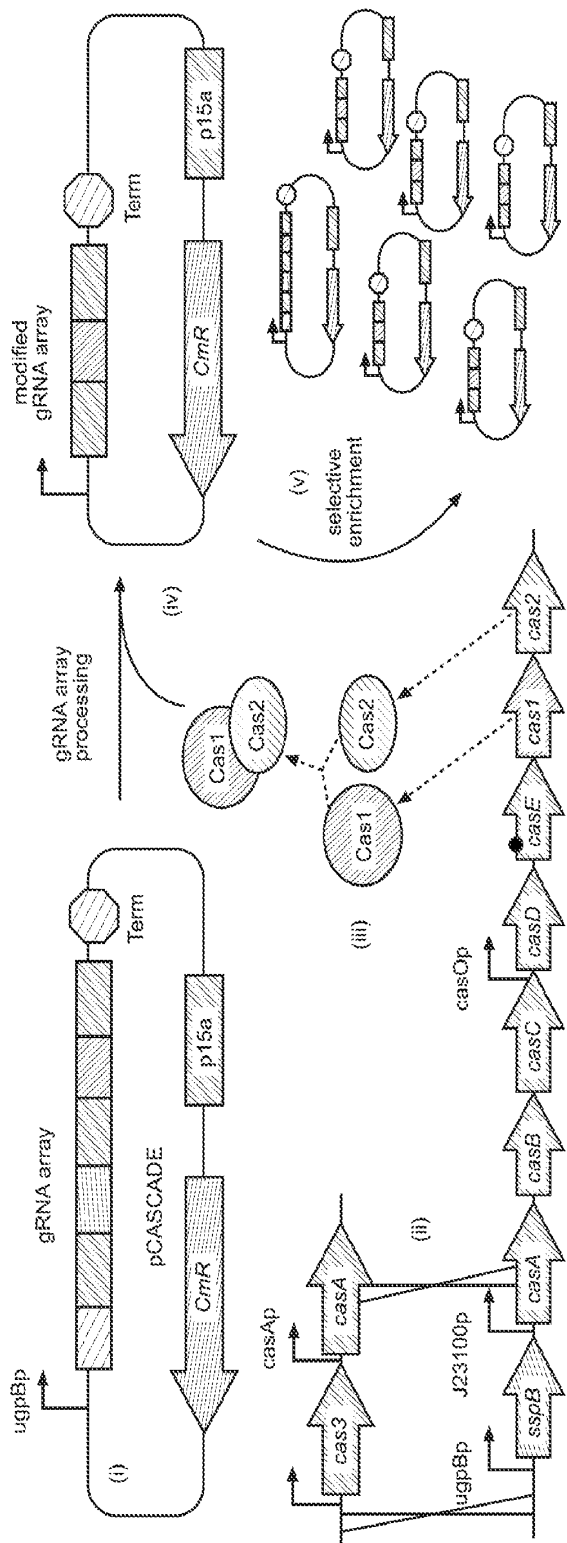


FIG. 1A

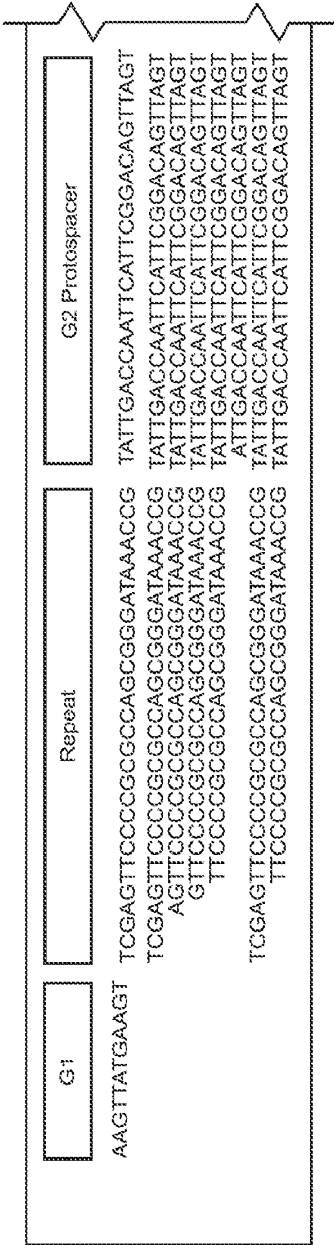


FIG. 1B

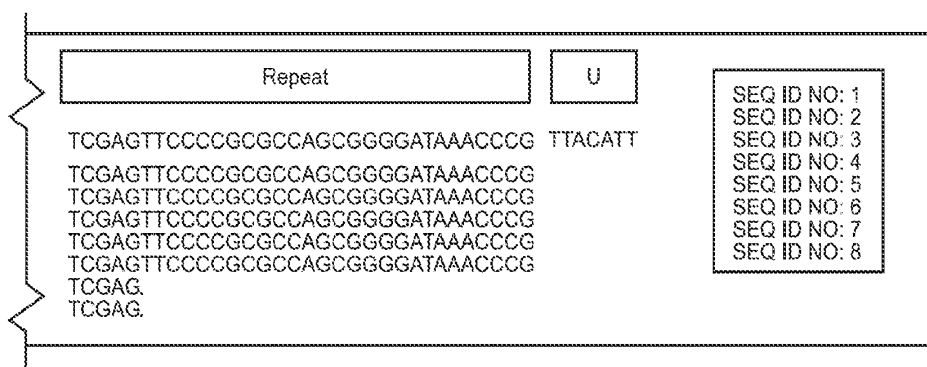


FIG. 1B
CON'T

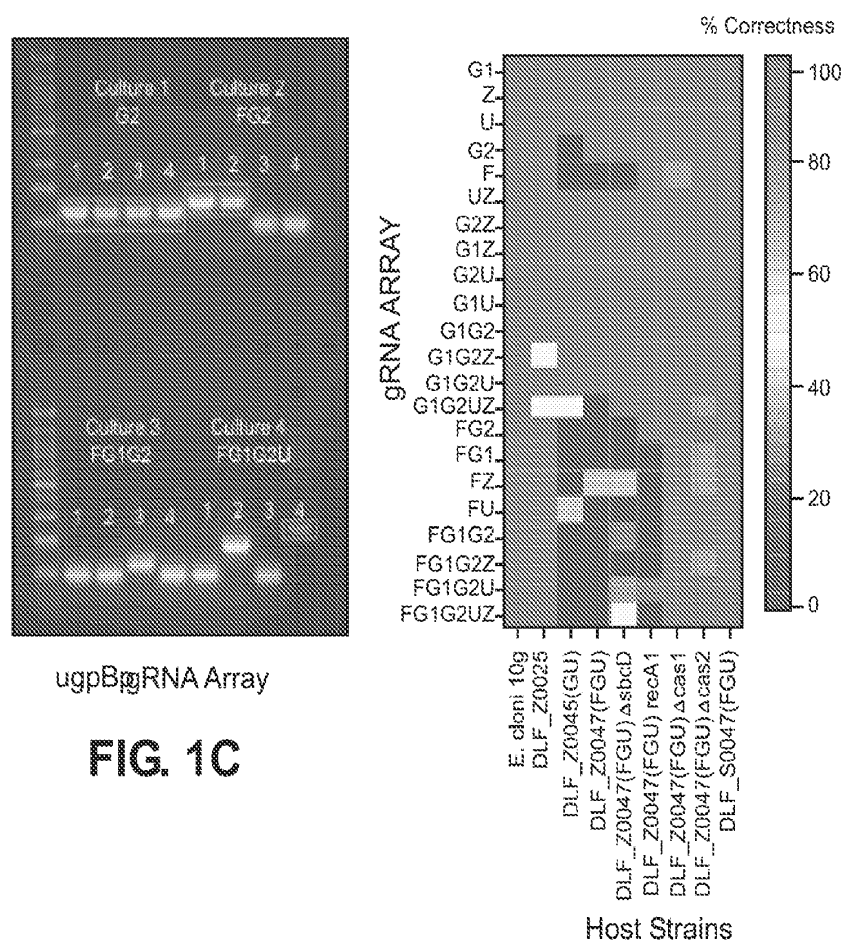


FIG. 1D

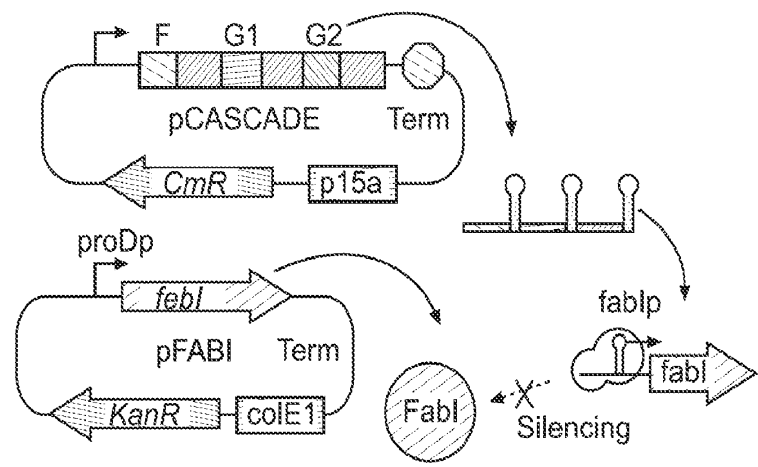


FIG. 1E

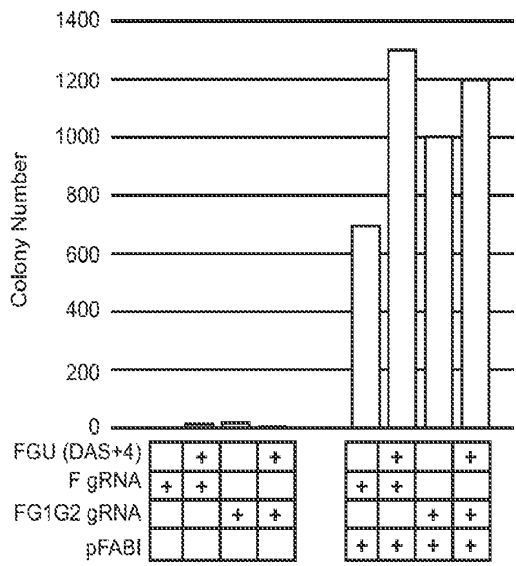


FIG. 1F

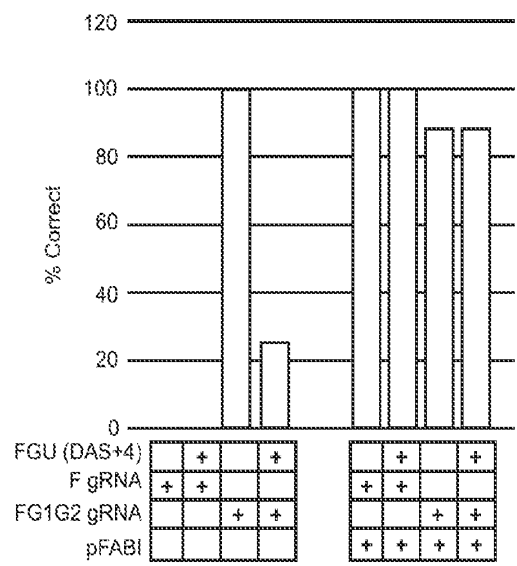


FIG. 1G

Plasmid	Insert	promoter	ori	Res	Addgene	Source
pCASCADE-G2	G2 guide	ugpBp	p15a	Cm	65817	Cit
pCASCADE-G1	G1 guide	ugpBp	p15a	Cm	71334	Ala
pCASCADE-F	F guide	ugpBp	p15a	Cm	66635	This study
pCASCADE-U	U guide	ugpBp	p15a	Cm	65818	Ala
pCASCADE-Z	Z guide	ugpBp	p15a	Cm	65825	Cit
pCASCADE-UZ	U, Z guide array	ugpBp	p15a	Cm	87153	Ala
pCASCADE-G2Z	G2, Z guide array	ugpBp	p15a	Cm	71338	Cit
pCASCADE-G1Z	G1, Z guide array	ugpBp	p15a	Cm	71337	Ala
pCASCADE-G1U	G1, U guide array	ugpBp	p15a	Cm	71339	Ala
pCASCADE-G2U	G2, U guide array	ugpBp	p15a	Cm	65819	Ala
pCASCADE-G1G2	G1, G2 guide array	ugpBp	p15a	Cm	71348	Ala
pCASCADE-G1G2U	G1, G2, U guide array	ugpBp	p15a	Cm	71343	Ala
pCASCADE-G1G2Z	G1, G2, Z guide array	ugpBp	p15a	Cm	71347	Ala

FIG. 2

Plasmid	Insert	promoter	ori	Res	Addgene	Source
pCASCADE-G1G2UZ	G1, G2, U, Z guide array	ugpBp	p15a	Cm	87152	Ala
pCASCADE-FG1	F, G1 guide array	ugpBp	p15a	Cm	71340	This study
pCASCADE-FG2	F, G2 guide array	ugpBp	p15a	Cm	71341	This study
pCASCADE-FU	F, U guide array	ugpBp	p15a	Cm	66636	This study
pCASCADE-FZ	F, Z guide array	ugpBp	p15a	Cm	71335	This study
pCASCADE-FG1G2	F, G, G2 guide array	ugpBp	p15a	Cm	71342	This study
pCASCADE-FG1G2Z	F, G, G2, Z guide array	ugpBp	p15a	Cm	66636	This study
pCASCADE-FG1G2U	F, G, G2, U guide array	ugpBp	p15a	Cm	66637	This study
pCASCADE-FG1G2UZ	F, G, G2, U, Z guide array	ugpBp	p15a	Cm	87148	This study
pFAB1	fab1	EM7	colE1	Kan	138659	This study

FIG. 2
CON'T

Strain	Genotype	Source
E. cloni 10G	F- <i>mcrA</i> $\Delta(mrr-hsdRMS-mcrBC)$ <i>endA1 recA1</i> $\Phi 80dlacZ\Delta M15$ $\Delta lacX74 araD139 \Delta(ara, leu)7697galU galK rpsL mupG \lambda^- tonA$ (StrR)	Lucigen
DLF_Z0025	F-, λ^- , $\Delta(araD-araB)567$, <i>lacZ4787(del)(::rrnB-3)</i> , <i>rph-1</i> , $\Delta(rhaD-rhaB)568$, <i>hsdR514</i> , $\Delta ackA-pta$, $\Delta poxB$, $\Delta pflB$, $\Delta ldhA$, $\Delta adhE$, $\Delta iclR$, $\Delta arcA$, $\Delta sspB::frt$, $\Delta cas3::ugpBp-sspB-J23100p-casA$	Cit
DLF_Z0045	DLF_Z0025, <i>gltA-DAS4::zeoR</i> , <i>udhA-DAS4::bsdR</i>	Cit
DLF_Z0047	DLF_Z0045, <i>fabI-DAS4::gentR</i>	Ala
DLF_Z0047, <i>recA1</i>	DLF_Z0047, <i>recA1::ampR</i>	This study
DLF_Z0047, <i>AsbcD</i>	DLF_Z0047, <i>AsbcD::ampR</i>	This study
DLF_Z0047, <i>Acas1</i>	DLF_Z0047, <i>Acas1::purR</i>	This study
DLF_Z0047, <i>Acas2</i>	DLF_Z0047, <i>Acas2::purR</i>	This study
DLF_S0025	F-, λ^- , $\Delta(araD-araB)567$, <i>lacZ4787(del)(::rrnB-3)</i> , <i>rph-1</i> , $\Delta(rhaD-rhaB)568$, <i>hsdR514</i> , $\Delta ackA-pta$, $\Delta poxB$, $\Delta pflB$, $\Delta ldhA$, $\Delta adhE$, $\Delta iclR$, $\Delta arcA$, $\Delta sspB::frt$, $\Delta cas3::ugpBp-sspB-yibDp-casA$	This study
DLF_S0047	DLF_S0025, <i>fabI-DAS4::gentR</i> , <i>gltA-DAS4::zeoR</i> , <i>udhA-DAS4::bsdR</i>	This study

Res - resistance marker, Cm- chloramphenicol, bsd - blasticidin, zeo- zeocin, gent - gentamicin, ampR- ampicillin, purR - puromycin.

FIG. 3

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sgRNA/Primer Name	Sequence	Template
fabI	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCG</i> (SEQ ID NO: 9)	
fabI-FOR	GTITATCTGTTTCGTATCGAGTTCCCCGCGCCAGCGGGGA TAAACCGAAAAAAAACCCC(SEQ ID NO: 10)	pCASCADE control
fabI-REV	GGTTATTATAATCAACGTTTATCCCCGCTGGCGCGGG GAACTCGAGGTGGTACCAGATC(SEQ ID NO: 11)	
FG1	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGAAAAGCATATAATGCGTAAAAGTT ATGAAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCG</i> (S EQ ID NO: 12)	
gltA1-FOR	GCGCCAGCGGGGATAAACCGAAAAGCATATAATGCG(SEQ ID NO: 13)	pCASCADE-gltA1
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SEQ ID NO: 14)	
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SEQ ID NO: 15)	pCASCADE-fabI
fabI-REV	CGGTTTATCCCCGCTGGCGCGGGGAAGTCGATACGAAC AGATAAACGGTTATTATAATC(SEQ ID NO: 16)	
FG2	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGTATTGACCAATTCATTCGGGACAGT TATTAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCG</i> (S EQ ID NO: 17)	
gltA2-FOR	GCGCCAGCGGGGATAAACCGTATTGACCAATTCATTC(SEQ ID NO: 18)	pCASCADE-gltA2
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SEQ ID NO: 19)	

FIG. 4

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sgRNA/Primer Name	Sequence	Template
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SEQ ID NO: 20)	pCASCADE-fabI
fabI-REV	CGGTTTATCCCCGCTGGCGCGGGGAACTCGATACGAACAGATAAACGGTTATTATAATC(SEQ ID NO: 21)	
FU	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTATAATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGTTACCACTCTGTTGCTTTTATGTATA AGAATCGAGTTCCCCGCGCCAGCGGGGATAAACCG</i> (SEQ ID NO: 22)	
udhA-FOR	GCGCCAGCGGGGATAAACCGTTACCACTCTGTTG(SEQ ID NO: 23)	pCASCADE-udhA
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SEQ ID NO: 24)	
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SEQ ID NO: 25)	pCASCADE-fabI
fabI-REV	CGGTTTATCCCCGCTGGCGCGGGGAACTCGATACGAACAGATAAACGGTTATTATAATC(SEQ ID NO: 26)	
FZ	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTATAATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGCTCGTAAAAGCAGTACAGTGCACC GTAAGATCGAGTTCCCCGCGCCAGCGGGGATAAACCG</i> (SEQ ID NO: 27)	
zwf-FOR	GCGCCAGCGGGGATAAACCGCTCGTAAAAG(SEQ ID NO: 28)	pCASCADE-zwf
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SEQ ID NO: 29)	
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SEQ ID NO: 30)	pCASCADE-fabI
fabI-REV	CGGTTTATCCCCGCTGGCGCGGGGAACTCGATACGAACAGATAAACGGTTATTATAATC(SEQ ID NO: 31)	

FIG. 4
CON'T 1

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sgRNA/Primer Name	Sequence	Template
FG1G2	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGAAAAGCATATAATGCGTAAAAGTT ATGAAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCGT ATTGACCAATTCATTTCGGGACAGTTATTAGTTCGAGTTC CCCCGCGCCAGCGGGGATAAACCG</i> (SEQ ID NO: 32)	
gltA2-FOR	<i>GCGCCAGCGGGGATAAACCGTATTGACCAATTCATT</i> (SEQ ID NO: 33)	pCASCADE-gltA2
pCASCADE-REV	<i>CTTGCCCGCCTGATGAATGCTCATCCGG</i> (SEQ ID NO: 34)	
pCASCADE-FOR	<i>CCGGATGAGCATTTCATCAGGCGGGCAAG</i> (SEQ ID NO: 35)	pCASCADE-FG1
gltA1-REV	<i>CGGTTTATCCCCGCTGGCGCGGGGAACTCGAACTTCAT AACTTTTAC</i> (SEQ ID NO: 36)	
FG1G2U	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGAAAAGCATATAATGCGTAAAAGTT ATGAAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCGT ATTGACCAATTCATTTCGGGACAGTTATTAGTTCGAGTTC CCCCGCGCCAGCGGGGATAAACCGTTACCATTTCTGTTGCT TTTATGTATAAGAATCGAGTTCCCCGCGCCAGCGGGGAT AAACCG</i> (SEQ ID NO: 37)	
gltA2-FOR	<i>GCGCCAGCGGGGATAAACCGTATTGACCAATTCATT</i> (SEQ ID NO: 38)	pCASCADE-udhA
pCASCADE-REV	<i>CTTGCCCGCCTGATGAATGCTCATCCGG</i> (SEQ ID NO: 39)	
pCASCADE-FOR	<i>CCGGATGAGCATTTCATCAGGCGGGCAAG</i> (SEQ ID NO: 40)	pCASCADE-FG1G2
gltA1-REV	<i>CGGTTTATCCCCGCTGGCGCGGGGAACTCGAACTTCAT AACTTTTAC</i> (SEQ ID NO: 41)	

FIG. 4
CON'T 2

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sgRNA/Primer Name	Sequence	Template
FG1G2Z	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGAAAAGCATATAATGCGTAAAAGTT ATGAAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCGT ATTGACCAATTCATTTCGGGACAGTTATTAGTTCGAGTTC CCCGCGCCAGCGGGGATAAACCGCTCGTAAAAGCAGTA CAGTGCACCGTAAGATCGAGTTCCCCGCGCCAGCGGGG ATAAACCG</i> (SEQ ID NO: 42)	
gltA2-FOR	GCGCCAGCGGGGATAAACCGTATTGACCAATTCATT(CSE Q ID NO: 43)	pCASCADE-zwf
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SAQ ID NO: 44)	
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SAQ ID NO: 45)	pCASCADE-FG1G2
gltA1-REV	CGGTTTATCCCCGCTGGCGCGGGGAACCTCGAACTTCAT AACTTTTAC(SAQ ID NO: 46)	
FG1G2UZ	<i>TCGAGTTCCCCGCGCCAGCGGGGATAAACCGTTGATTAT AATAACCGTTTATCTGTTTCGTATCGAGTTCCCCGCGCCA GCGGGGATAAACCGAAAAGCATATAATGCGTAAAAGTT ATGAAGTTCGAGTTCCCCGCGCCAGCGGGGATAAACCGT ATTGACCAATTCATTTCGGGACAGTTATTAGTTCGAGTTC CCCGCGCCAGCGGGGATAAACCGTTACCATTCCTGTTGCT TTTATGTATAAGAATCGAGTTCCCCGCGCCAGCGGGGAT AAACCGCTCGTAAAAGCAGTACAGTGCACCGTAAGATC GAGTTCCCCGCGCCAGCGGGGATAAACCG</i> (SEQ ID NO: 47)	
zwf-FOR	GCGCCAGCGGGGATAAACCGCTCGTAAAAG(SAQ ID NO: 48)	pCASCADE-zwf
pCASCADE-REV	CTTGCCCGCCTGATGAATGCTCATCCGG(SAQ ID NO: 49)	
pCASCADE-FOR	CCGGATGAGCATTTCATCAGGCGGGCAAG(SAQ ID NO: 50)	pCASCADE-FG1G2U
udhA-REV	CGGTTTATCCCCGCTGGCGCGGGGAACCTCGATTCTTATA CATAAAAGC(SAQ ID NO: 51)	

FIG. 4
CON'T 3

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tetA-sacB Cassette
<p>TCCTAATTTTGTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAAA AGTGAAATGAATAGTTTCGACAAAGATCGCATTGGTAATTACGTTACTCGATGCCATGGGGATTGGCC TTATCATGCCAGTCTTGCCAACGTTATTACGTGAATTTATGCTTCGGAAGATATCGCTAACCACTTT GGCGTATTGCTTGCACTTTATGCGTTAATGCAGGTTATCTTTGCTCCTTGGCTTGGAAAAATGTCTGA CCGATTTGGTCGGCGCCAGTGTGTTGTTGTCATTAAATAGGCGCATCGCTGGATTACTTATTGCTGG CTTTTCAAGTGGCGCTTTGGATGCTGTATTTAGGCCGTTTGCCTTCAGGGATCACAGGAGCTACTGGG GCTGTGCGGGCATCGGTCATTGCCGATACCACCTCAGCTTCTCAACGCGTGAAGTGGTTGCGTTGGT TAGGGGCAAGTTTTGGGCTTGGTTTAATAGCGGGGCTATTATTGGTGGTTTTGCAGGAGAGATTTT ACCGCATAGTCCCTTTTTATCGCTGCGTTGCTAAATATTGTCACCTTTCCTTGTGGTTATGTTTTGGTT CCGTGAAACCAAAAATACACGTGATAATACAGATACCGAAGTAGGGGTTGAGACGCAATCGAATTC GGTATACATCACTTTATTTAAAACGATGCCCATTTTGTGATTATTTATTTTTCAGCGCAATTGATAG GCCAAATTCCCGCAACGGTGTGGGTGCTATTTACCGAAAAATCGTTTTGGATGGAATAGCATGATGGT TGGCTTTTCATTAGCGGGTCTTGGTCTTTTACACTCAGTATTCCAAGCCTTTGTGGCAGGAAGAATAG CCACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGATTTATTGCAGATAGTAGTGCATTTGCCTT TTTAGCGTTTATATCTGAAGGTTGGTTAGTTTCCCTGTTTTAATTTTATGCGTGGTGGTGGGATCG CTTTACCTGCATTACAGGGAGTGATGCTCTATCCAAACAAAGAGTCATCAGCAAGGTGCTTTACAGGG ATTATTGGTGAGCCTTACCAATGCAACCGGTGTTATTGGCCCACTTACTGTTTGTGTTATTTATAATC ATTCACTACCAATTTGGGATGGCTGGATTGGATTATTGGTTTAGCGTTTACTGTATTATTATCCTG CTATCGATGACCTTCATGTTAACCCTCAAGCTCAGGGGAGTAAACAGGAGACAAGTGCTTAGTTAT TTCGTACCAAATGATGTTATCCGCGAAATATAATGACCCTCTTGATAACCCAAGAGCATCACATA TACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTCTGAATTGTGATTAATAAAGG CAACTTTATGCCCATGCAACAGAACTATAAAAAATACAGAGAATGAAAAAGAAACAGATAGATTTT TTAGTTCTTTAGGCCCGTAGTCTGCAAAATCCTTTTATGATTTTCTATCAAACAAAAGAGGAAAAATAG ACGAGTTGCAATCCAAACGAGAGTCTAATAGAATGAGGTGCGAAAAGTAAATCGCGCGGGTTTGTTA CTGATAAAGCAGGCAAGACCTAAATGTGTAAAGGGCAAGGTGATATACTTTGGCGTCAACCTTAC ATATTTTAGGTCTTTTTTTATTGTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGCTGGAAGAAG CAGACCGCTAACACAGTACATAAAAAAGGAGACATGAACGATGAACATCAAAAAAGTTTGCAAAAC AAGCAACAGTATTAACCTTTACTACCGCACTGCTGGCAGGAGGCGCAACTCAAGCGTTTGCGAAAG AAACGAACCAAAAGCCATATAAGGAAACATACGGCATTTCCCATATTACACGCCATGATATGCTGC AAATCCCTGAACAGCAAAAAAATGAAAAATATCAAGTTCCCTGAGTTCGATTTCGTCCACAATTAATA ATATCTCTTCGCAAAAGGCCTGGACGTTTGGGACAGCTGGCCATTACAAAACGCTGACGGCACTGT CGCAAACTATCACGGCTACCACATCGTCTTTGCATTAGCCGGAGATCCTAAAAATGCGGATGACACA TCGATTTACATGTTCTATCAAAAAGTCGGCGAAACTTCTATTGACAGCTGGAAAAACGCTGGCCGCG TCTTTAAAGACAGCGACAAATTCGATGCAAATGATTCTATCCTAAAAGACCAACACAAGAATGGT CAGGTTACGCCACATTTACATCTGACGGAAAAATCCGTTTATTCTACACTGATTTCTCCGGTAAACA TTACGGCAAAACAAACACTGACAACCTGCACAAGTTAACGTATCAGCATCAGACAGCTCTTTGAACAT CAACGGTGTAGAGGATTATAAATCAATCTTTGACGGTGACGGAAAAACGTATCAAAATGTACAGCA GTTTCATCGATGAAGGCAACTACAGCTCAGGCGACAACCATACGCTGAGAGATCCTCACTACGTAGA AGATAAAGGCCACAAATACTTAGTATTTGAAGCAAACACTGGAAGTGAAGATGGCTACCAAGGCGA AGAATCTTTATTTAACAAAGCATACTATGCAAAAAGCACATCATTCTTCCGTCAAGAAAGTCAAAAA CTTCTGCAAAAGCGATAAAAAACGCACGGCTGAGTTAGCAAAACGGCGCTCTCGGTATGATTGAGCTA AACGATGATTACACACTGAAAAAAGTGATGAAACCGCTGATTGCATCTAACACAGTAACAGATGAA ATTGAACGCGCGAACGTCCTTTAAATGAACGGCAATGGTACCTGTTCACTGACTCCCGCGGATCAA AAATGACGATTGACGGCATTACGTCTAACGATATTTACATGCTTGGTTATGTTTCTAATTCCTTAACT GGCCCATACAAGCCGCTGAACAAAACTGGCCTTGTGTTAAAAATGGATCTTGATCTTAACGATGTAA CCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAATGTCGTGATTACAAGCTATAT GACAAACAGAGGATTCTACGCAGACAAACAATCAACGTTTGGCGCAAGCTTCTGCTGAACATCAA AGGCAAGAAAAACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAATTAACAGTTAACAAATA AAAACGCAAAAAGAAAATGCCGATATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCC TGATTACGGCTGTCTATGTGTGACTGTTGAGCTGTAACAAGTTGTCTCAGGTGTTCAATTTCAATGTT TAGTTGCTTTGTTTTACTGGTTTCACTGTTCTATTAGGTGTTACATGCTGTTTCATCTGTTACATTGTC GATCTGTTTCATGGTGAACAGCTTTAAATGCACCAAAAACTCGTAAAAGCTCTGATGTATCTATCTTT TTTACACCGTTTTTCATCTGTGCATATGGACAGTTTCCCTTTGAT(SEQ ID NO: 52)</p>

FIG. 5

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recA1::ampR

CCATCTCTACCGGTTTCGCTTTCACTGGATATCGCGCTTGGGGCAGGTGGTCTGCCGATGGGGCCGTAT
 CGTCGAAATCTACGGACCGGAATCTTCCGGTAAAACACGCTGACGCTGCAGGTGATCGCCGCAGC
 GCAGCGTGAAGGTAAAACCTGTGCGTTTATCGATGCTGAACACGCGCTGGACCCAATCTACGCACG
 TAAACTGGGCGTCGATATCGACAACCTGCTGTGCTCCCAGCCGACACCGGCGAGCAGGCACTGGA
 AATCTGTGACGCCCTGGCGCGTTCTGGCGCAGTAGACGTTATCGTCGTTGACTCCGTGGCGGCACTG
 ACGCCGAAAAGCGGAAATCGAAGGCGAAATCGATGACTCTCACATGGGCCTTGCGGCACGTATGATG
 AGCCAGGCGATGCGTAAGCTGGCGGGTAACCTGAAGCAGTCCAACACGCTGCTGATCTTCATCAAC
 CAGATCCGTATGAAAATTGGTGTGATGTTCCGTAACCCGGAACCACTACCGGTGGTAACGCGCTG
 AAATTCTACGCCTCTGTTCTGCTCTGACATCCGTCGTATCGGCGCGGTGAAAGAGGGGCGAAAACGTGG
 TGGGTAGCGAAACCCGCGTGAAAGTGGTGAAGAACAAAATCGCTGCGCCGTTTAAACAGGCTGAAT
 TCCAGATCCTCTACGGCGAAGGTATCAACTTCTACGGCGAACTGGTTGACCTGGGCGTAAAGAGA
 AGCTGATCGAGAAAGCAGGCGCGTGGTACAGCTACAAAGGTGAGAAGATCGGTCAGGGTAAAGCG
 AATGCGACTGCCTGGCTGAAAGATAACCCGGAACCCGCGAAGAGATCGAGAAGAAAGTACGTGA
 GTTGCTGCTGAGCAACCCGAACCTCAACGCCGATTCTCTGTAGATGATAGCGAAGGCGTAGCAGA
 AACTAACGAAGATTTTAAATGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCCTGAATGATA
 TCAAGCTTGAATTCTGTTGGTTCATCCCGTGGGCATTGCATAGGGATAACAGGGTAATCTAAATACAT
 TCAATATCTATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG
 AATATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTCGCGCATTTTGCCCTCTCTGTTTTT
 GCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCCGAAGATCAGTTGGGTGCACGTGTGGGTTAC
 ATCGAACTGGACCTCAACAGCGGTAAAGATTCTTGAGAGTTTTCGCCCCGAAGAACGTTTCCCAATGA
 TGAGCACTTTTAAAGTTCTGCTCTGTGGCGCGGTATTATCCCGTATTGACGCCCGGGCAAGAGCAACT
 CGGTGCGCGCATACACTATTCTCAGAAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTT
 ACGGACGGCATGACAGTACGCGAATTATGCAGCGCTGCCATAACCATGAGTGATAACACGGCGGCC
 AACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTTACCGCTTTTTTGCACAACATGGGTGATC
 ATGTAACCTCGCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA
 CCACGATGCCTGTAGCTATGGCAACAACGTTGCGCAAACTCTTAACTGGCGAACTTCTTACTCTCGC
 TTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
 CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCCCAGCGGTATTATT
 GCAGCCCTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGCCAGOCA
 ACTATGGACGAACGTAATCGCCAGATCGCTGAGATAGGTGCCCTCCCTGATTAAAGCATTTGGTAATAAC
 CAAGCATCTCGTCTTTGTTGATACACAAGGGTCGCATCTGCGGCCCTTTTGCTTTTTTAAAGTTGTAAG
 GATATGCCATGACAGAATCAACATCCCGTCGCGCGGCATATGCTCGCTGTTGGATCGTGCGGTACG
 CATTCTGGCGGTGCGCGATCACAGTGAGCAAGAAGTGGACGTAAACTCGCGGCACCGATTATGGG
 CAAAAATGCCCCAGAAGAGATTGATGCTACGGCAGAAGATTACGAGCGCGTTATTG(SEQ ID NO: 53)

FIG. 5
CON'T 1

ΔsbcD::ampR

TGATTGCAGTCCAGTTACGCTGGAGTCTGAGGCTCGTCCTGAATGATATCAAGCTTGAATTCGTTGG
TTCATCCCGTGGGCATTGCATAGGGATAACAGGGTAATCTAAATACATTCAAATATCTATCCGCTCA
TGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAATATGAGTATTCAACATT
TCCGIGTCGCCCCTATTCCCTTTTTGCGGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGG
TGAAAGTAAAAGATGCCGAAGATCAGTTGGGTGCACGTGTGGGTTACATCGAACTGGACCTCAACA
GCGGTAAGATTCTTGAGAGTTTTTCGCCCCGAAGAACGTTTCCCAATGATGAGCACTTTTAAAGTTCT
GCTCTGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCCGCGCATACACTAT
TCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGACGGCATGACAGTA
CGCGAATTATGCAGCGCTGCCATAACCATGAGTGATAACACGGCGGCCAACTTACTTCTGACAACG
ATCGGAGGACCGAAAGGAGCTTACCGCTTTTTTGCACAACATGGGTGATCATGTAACCTCGCCTTGATC
GTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAGATGCCTGTAGCTA
TGGCAACAACGTTGCGCAAACTCTTAACTGGCGAACTTCTTACTCTCGCTTCCCGGCAACAATTAAT
AGACTGGATGGAGCGCGATAAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGCTGGCTGGTTT
ATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCCCGCGGTATTATTGCAGCCCTGGGGCCAGATG
GTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGAGCCAGGCAACTATGGAACGAACGTAATC
GCCAGATCGCTGAGATAGGTGCCTCCCTGATTAAGCATTGGTAATAACCAGGCAT(SEQ ID NO: 54)

FIG. 5
CON'T 2

Δcas3::Term-ugpBp-sspB-TZ yibDp-casA

TATGAGCAGCATCGAAAAATAGCCCGCTGATATCATCGATAACTAAAAAACAGGGAGGCTATT
ACCAGGCATCAAAATAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGT
CGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCGCGTTTATATCTTT
CTGACACCTTACTATCTTACAAATGTAAACAAAAAGTTATTTTCTGTAATTCGAGCATGTCATGTTA
CCCCGCGAGCATAAAACGCGTGTGTAGGAGGATAATCTATGGATTTGTCACAGCTAACACCCACGTC
GTCCCTATCTGCTGCGTGCATTCTATGAGTGGTTGCTGGATAACCAGCTCACGCCGCACCTGGTGGT
GGATGTGACGCTCCCTGGCGTGCAGGTTCTATGGAATATGCGCGTGACGGGCAATCGTACTCAAC
ATTGCGCCGCGTGCTGTCGGCAATCTGGAAGTGGCGAATGATGAGGTGCGCTTTAACGCGCGCTTTG
GTGGCATTCCGCGTCAGGTTTCTGTGCCGCTGGCTGCCGTGCTGGCTATCTACGCCCGTAAAAATGG
CGCAGGCACGATGTTTGAGCCTGAAGCTGCCTACGATGAAGATACCAGCATCATGAATGATGAAGA
GGCATCGGCAGACAACGAAACCGTTATGTCGGTTATTGATGGCGACAAGCCAGATCACGATGATGA
CACTCATCTGACGATGAACCTCCGCAGCCACCACGCGGTGGTCGACCGGCATTACGCGTTGTGAAG
TAATCGACCTAGCATAACCCCGCGGGGCCCTCTCGGGGGTCTCGCGGGGTTTTTGTGTAAGAAGC
TTCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTGCTGCG
GCCGGGTGAGGTATGATTTAAATGGTCAGTAACGGGTCTTGAGGGGTTTTTGTCCACAGCTAACACC
ACGTCGTCCCTATCTGCTGCCCTAGGTCTATGAGTGGTTGCTGGATAACGTGCGTAATTGTGCTGATC
TCTTATATAGCTGCTCTCATTATCTCTCTACCCTGAAGTGACTCTCTCACCTGTAAAAATAATATCTC
ACAGGCTTAATAGTTTCTTAATACAAAGCCTGTAAAAACGTCAGGATAACTTCTATATTCAGGGAGAC
CACAACGGTTTTCCCTCTACAAATAATTTTGTTTAACTTTTGAAGGAGAACAAATGAATTTGCTTATTG
ATAACTGGATCCCTGTACGCCCGCGAAACGGGGGGAAGTCCAAATCATAAATCTGCAATCGCTAT
ACTGCAGTAGAGATCAGTGGCGATTAAAGTTTGCCCCGTGACGATATGGAACTGGCCGCTTTAGCACT
GCTGGTTTGATTGGGCAAATTATCGCCCCGGCAAAAGATGACGTTGAATTTGACATCGCATAATG
AATCCGCTCACTGAAGATGAGT(SEQ ID NO: 59)

FIG. 5
CON'T3