

Supporting Information for DOI 10.1002/biot.201600053

## **CRISPR-based genome editing and expression control systems in *Clostridium acetobutylicum* and *Clostridium beijerinckii***

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## Supporting information, Table S1: Plasmids and strains used in this study

Strains or plasmids	Description <sup>a</sup>	Source or reference <sup>b</sup>
<b>Strains</b>		
<i>C. acetobutylicum</i> ATCC 8241	Type II restriction endonuclease Cac8241 deletion	This study
<i>C. beijerinckii</i> NCIMB 8052	Wild type	NCIMB
<i>C. acetobutylicum</i> ATCC 824	Wild type	ATCC
<i>E. coli</i> DH5α	Commercial transformation host	GIBCO BRL, Life Technologies
<b>Plasmids</b>		
pIMP1-P <sub>ptb</sub>	pIM13,MLS <sup>R</sup> , <i>ptb</i> promoter,ColE1 origin , Amp <sup>R</sup> , <i>E. coli</i> - <i>Clostridium</i> shuttle vector	Offered by Prof.Eleftherios T.Papoutsakis [1]
pXY1	pCB102,MLS <sup>R</sup> ,P <sub>thl</sub> promoter,ColE1 origin , Amp <sup>R</sup> , <i>E.coli</i> – <i>Clostridium</i> shuttle vector	
pLQ-donor	Derived from pIMP1-P <sub>ptb</sub> , homologous arms( <i>pyrE</i> ) ( <i>EcoRV</i> was added between the two 1-kb arms)	This study
pLQ-sgRNA	Derived from pIMP1-P <sub>ptb</sub> , pJ23119, sgRNA- <i>pyrE</i>	This study
pLQ-Cas9	Derived from pIMP1-P <sub>ptb</sub> , <i>cas9</i>	This study
pLQ-Cas9n	Derived from pIMP1-P <sub>ptb</sub> , <i>cas9</i> with D10A mutation	This study
pLQ-sgRNA-Cas9	Derived from pIMP1-P <sub>ptb</sub> , pJ23119, sgRNA- <i>pyrE</i> , <i>cas9</i>	This study
pLQ-sgRNA-Cas9n	Derived from pIMP1-P <sub>ptb</sub> , pJ23119, sgRNA- <i>pyrE</i> , <i>cas9</i> with D10A mutation	This study
pCASclos- <i>pyrE</i>	Derived from pLQ-sgRNA-Cas9, homologous arms ( <i>EcoRV</i> was added between the two 1-kb arms)	This study
pNICKclos1.0- <i>pyrE</i>	Derived from pLQ-sgRNA-Cas9n, homologous arms ( <i>EcoRV</i> was added between the two 1-kb arms)	This study
pNICKclos1.0- <i>adc</i>	Derived from pNICKclos1.0- <i>pyrE</i> , sgRNA- <i>adc</i> (824), homologous arms ( <i>EcoRV</i> was added between the two 1-kb arms)	This study
pXY1-Cas9n	Derived from pXY1, <i>cas9</i> with D10A mutation, P <sub>thl</sub> -Cas9 nickase	This study
pNICKclos2.0- <i>agrA</i>	Derived from pXY1-Cas9n, pJ23119, sgRNA- <i>agrA</i> , homologous arms ( <i>PstI</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>adc</i>	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>adc</i> (8052), homologous arms ( <i>PstI</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>xylR</i>	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>xylR</i> , homologous arms ( <i>PstI</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>araR</i>	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>araR</i> , homologous arms ( <i>EcoRV</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>cbei3923</i>	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>cbei3923</i> , homologous arms ( <i>PstI</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>cbei4495</i>	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>cbei4495</i> , homologous arms ( <i>PstI</i> was added between the two 1-kb arms)	This study
pNICKclos2.0- <i>xylR</i> -ORF	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>xylR</i> , homologous arms (targeting the Open Reading Frame of <i>xylR</i> gene with two 1.2-kb arms)	This study
pNICKclos2.0- <i>xylR</i> -HA150	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>xylR</i> , homologous arms ( <i>PstI</i> was added between the two 0.15-kb arms)	This study
pNICKclos2.0- <i>xylR</i> -HA200	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>xylR</i> , homologous arms ( <i>PstI</i> was added between the two 0.2-kb arms)	This study
pNICKclos2.0- <i>xylR</i> -HA500	Derived from pNICKclos2.0- <i>agrA</i> , pJ23119, sgRNA- <i>xylR</i> , homologous arms ( <i>PstI</i> was added between the two 0.5-kb arms)	This study
pdCASclos- <i>cac2071</i>	Derived from pIMP1-P <sub>ptb</sub> , pJ23119, sgRNA- <i>spo0A</i> (824), Cas9 with D10A and H840A mutations	This study
pdCASclos- <i>cbei1712</i>	Derived from pdCASclos- <i>cac2071</i> , pJ23119, sgRNA- <i>spo0A</i> (8052)	This study

<sup>a</sup> pIM13, Gram-positive origin of replication in *C. beijerinckii* and *C. acetobutylicum*; MLS<sup>R</sup>, macrolide-lincosamide-streptogramin B

resistance; Amp<sup>R</sup>, ampicillin resistance; pCB102, Gram-positive origin of replication in *C. beijerinckii* and *C. acetobutylicum*.

<sup>b</sup> NCIMB, National Collection of Industrial, Marine and Food Bacteria; ATCC, American Type Culture Collection, Manassas, VA

**Supporting information, Table S2: Oligonucleotides used in this study**

<b>Oligos</b>	<b>Sequence (5'→3')</b>
Cas9-up	tccccccggg atggataagaaataactcaataggct
Cas9-dn	tccccccgggtcagtcacctcctagctgactcaaa
Cas9n-up	tccccccgggatggataagaaataactcaataggcttagctatcggcacaaatagc
Cas9n-dn	tccccccgggtcagtcacctcctagctgactcaaatcaat
sgRNA- <i>pyrE</i> -1-1	gtcctaggtataatactagttatacttaaatcacaggctggttttagagctagaaatagc
sgRNA- <i>pyrE</i> -1-2	gaggtcaatctatgaaaatgcgattaagcttggctgcaggctcctaggtataatactagt
sgRNA- <i>pyrE</i> -2	agtggcaccgagtcggtgcttttttgcggccgcattcttatgaaaattcaagtgctct
<i>pyrE</i> -up-1	agagcacttgaaatttcataagaatgcggccgcacaaaaagcaccgactcggtgccact
<i>pyrE</i> -up-2	ggtacatcagtatacgaacaatgccgatatcaggttgatgtaaagggaaatcataata
<i>pyrE</i> -dn-1	tattatgattccctttacatcaacctgatatcggcattgtttcgtatactgatgtacc
<i>pyrE</i> -dn-2	tttttagaaaattatttatattttatactgcagctcgaggatgtttatacagttactat
P1	taaggggattcaaccaagtttactttataaggat
P2	aattattagataatctttcatcagcaaaatcaccacaaca
<i>pyrE</i> -seq	gtagctatggcacttgataa
<i>adc</i> (824)-1-1	taggtataataactagtaaaattgatgagcccttagtcggttttagagctagaaatagcaa
<i>adc</i> (824)-1-2	gattaagcttggctgcagttgacagctagctcagtcctaggtataataactagtaaaattg
<i>adc</i> (824)-2	aagaacaagatattatagaccagcggccgcacaaaaagcaccgactcggtgcc
<i>adc</i> (824)-3	ggcaccgagtcggtgcttttttgcggccgctggtctataatatcttgttctt
<i>adc</i> (824)-4	gcattgccataatttcaaacctgatatcctaaaggctctggcacaacttt
<i>adc</i> (824)-5	aaagttgtgccagagccttaggatatcaggttgaaattatggcaatgc
<i>adc</i> (824)-6	tttttagaaaattatttatattttatactgcagctcgagggtggtcacgtagatgttact
P3	aagcactgtttttcgtctaagac
P4	cggaaacaccttctataaagggtactac
<i>adc</i> (824)-seq	ggtgacttttatgttaaaggatgaagtaat
<i>agrA</i> -1-1	cctaggtataataactagtaaaatgatccaagggttttagagctagaaatagca
<i>agrA</i> -1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataataactagtaaaat
<i>agrA</i> -2	ataatactattccttttattctgcggccgcacaaaaagcaccgactcggtgcc
<i>agrA</i> -3	ggcaccgagtcggtgcttttttgcggccgcagaataaaaggaatagtattat
<i>agrA</i> -4	tgtacaaatacaataaatcccctgcagttcagcagctagctgtatccca
<i>agrA</i> -5	tgggatacagctagctgctgaactgcaggggattattgtattgttaca
<i>agrA</i> -6	attattattttatcaatatattttgttaaaaactcgagtcacaaaatttctcttttacc
P5	ccaatgtagtttttctcctaataattct
P6	taaagcttggtacgtcatttgttga
<i>adc</i> (8052)-1-1	cctaggtataataactagtaaaatcattcaagggttacgagtttttagagctagaaatagca
<i>adc</i> (8052)-1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataataactagtaaaat
<i>adc</i> (8052)-2	atcaaaaaatgaaccttgaggtgcggccgcacaaaaagcaccgactcggtg
<i>adc</i> (8052)-3	caccgagtcggtgcttttttgcggccgcacctcaagggtcattttttgat
<i>adc</i> (8052)-4	ttcacaattcttggcttaccactgcagtagcataaaattgggtcttgca
<i>adc</i> (8052)-5	tgcaagaccaattttatgctactgcagtggttaagccaagaattttgtgaa
<i>adc</i> (8052)-6	attattattttatcaatatattttgttaaaaactcgagatagttgggaggttacacct
P7	cccagatataataaatgctggcg
P8	aggttgactacattatctgatagcc
<i>adc</i> (8052)-seq	ggtcaagctattccagtaaa
<i>agrA</i> -seq	agtaccctaagtattgttggtgatac
<i>xylR</i> -1-1	cctaggtataataactagtcgagtttagacataatagtgagtttttagagctagaaatagca
<i>xylR</i> -1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataataactagtcgagt
<i>xylR</i> -2	aattccattagaaccaggttttgcggccgcacaaaaagcaccgactcggtg
<i>xylR</i> -3	caccgagtcggtgcttttttgcggccgcacaaacctggttctaattggaatt
<i>xylR</i> -4	attattatgttcttattccactgcagcatactttcaataactttttc
<i>xylR</i> -5	gaaaaaagttattgaaagtatgctgcagtggaatagagaacataataaat
<i>xylR</i> -6	attattattttatcaatatattttgttaaaaactcgagaaaatcaagaacataaatct

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P9	ctatgattattaactgaaaaagcagca
P10	acataagaaatttgctcttaattcgg
<i>xylR</i> -seq	agcgaaggtattgtagaagatgtaa
<i>araR</i> -1-1	cctaggtataatactagtagatgatgagcagggagtttagagctagaaatagca
<i>araR</i> -1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataatactagttagta
<i>araR</i> -2	tccatcttctaagttagagatgctggccgcaaaaaagcaccgactcggtg
<i>araR</i> -3	caccgagtcggtgctttttgctggccgcctctcacttaagaagatgga
<i>araR</i> -4	aataattagtgagctgtacccgatatccaatatatgcagaattttcttc
<i>araR</i> -5	gaagaaaattctgcatatattggatatcggtacaagctcactaattatt
<i>araR</i> -6	attattattttatcaatatattttgttaaaaactcgagctgcatgtgtgtcccaaaa
P11	ttatacccttaaggggcacctagt
P12	ccgagtacaaggaatatcccat
<i>cbei3923</i> -1-1	cctaggtataatactagtataaatacatatcaaaacaggttttagagctagaaatagca
<i>cbei3923</i> -1-2	tgcagttgacagctagctcagtcctaggtataatactagtataaa
<i>cbei3923</i> -2	tatttacattaaattatttagagcggccgcaaaaaagcaccgactcggtg
<i>cbei3923</i> -3	caccgagtcggtgctttttgctggccgcctatgtcgatttaattattct
<i>cbei3923</i> -4	gtccaactattgaaaaagaattctgcagcggaaatgtttatgaaaaagat
<i>cbei3923</i> -5	atcttttcataaacatttccgctgcagaattctttcaataagttggac
<i>cbei3923</i> -6	attattattttatcaatatattttgttaaaaactcgagcatctaccttcttaattta
P13	caatgattttggttaataataactataaatgttgaa
P14	ttacaaatctttttaagctgtattataatcttca
<i>cbei3923</i> -seq	tgaatggaattttaacatcaaa
<i>cbei4495</i> -1-1	cctaggtataatactagtttcaaatactttctagaaatgttttagagctagaaatagca
<i>cbei4495</i> -1-2	tgcagttgacagctagctcagtcctaggtataatactagtttcaa
<i>cbei4495</i> -2	aattttcacttatataactggcggccgcaaaaaagcaccgactcggtg
<i>cbei4495</i> -3	caccgagtcggtgctttttgctggccgcagtatataagtatgaaaaatt
<i>cbei4495</i> -4	tcagatttaagtttaaagtcgctgcaggttgagggactatttcatttat
<i>cbei4495</i> -5	ataaatgaaatagtcctcaactgcagcggactttaaaactaaatctga
<i>cbei4495</i> -6	attattattttatcaatatattttgttaaaaactcgaggtattctatggctttttta
P15	tgaggttactattgatttttttacgcttaata
P16	atggcaattgtgttaggtttctt
<i>cbei4495</i> -seq	ttggctttaacacgat
<i>xylR</i> -ORF-1-1	cctaggtataatactagtcgagtttagacataatagtgagtttagagctagaaatagca
<i>xylR</i> -ORF-1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataatactagtcgagt
<i>xylR</i> -ORF-2	ctgcttggtctccagcaccaccaacaactggtgtatctagaaaaaagcaccgactcgg
<i>xylR</i> -ORF-3	ccgagtcggtgctttttctagatacaccagttgttggtggtgctggagaccaagcag
<i>xylR</i> -ORF-4	agagcagctttcaccactccaactttaaaatactcctctcact
<i>xylR</i> -ORF-5	agtcgagaggagtattttaagttggagtgggtgaaagctgctct
<i>xylR</i> -ORF-6	attattattttatcaatatattttgttaaaaactcgagtggttgtagtgcttattgat
P17	ggaaaagaaagactaattaagatcactggt
P18	aaatatattatccataaacatttatgccttcc
<i>xylR</i> -ORF-seq	ctccaaatgaagaattaaaagaagtatact
<i>xylR</i> -150-1-1	cctaggtataatactagtcgagtttagacataatagtgagtttagagctagaaatagca
<i>xylR</i> -150-1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataatactagtcgagt
<i>xylR</i> -150-2	ttcttcctcctgtagattccaacgatcttacatcttagaaaaaagcaccgactcgg
<i>xylR</i> -150-3	ccgagtcggtgctttttctagagatgtaagatcgttggaatctacaggaggaagaaa
<i>xylR</i> -150-4	attattatgttctctattccactgcagcactttcaataactttttc
<i>xylR</i> -150-5	gaaaaaagttattgaaagtatgctgcagtggaatagagaacataataaat
<i>xylR</i> -150-6	ttttatcaatatattttgttaaaaactcgagaaatagaatactttattatgccttct
<i>xylR</i> -200-1-1	cctaggtataatactagtcgagtttagacataatagtgagtttagagctagaaatagca
<i>xylR</i> -200-1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataatactagtcgagt
<i>xylR</i> -200-2	gcttttcaattctgtaatatgttagaaaactgtgtctagaaaaaagcaccgactcgg
<i>xylR</i> -200-3	ccgagtcggtgctttttctagacaacagtttctacaaatattacagaattgaaaagc
<i>xylR</i> -200-4	atttattatgttctctattccactgcagcactttcaataactttttc

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<i>xyIR</i> -200-5	gaaaaaagttattgaaagtatgctgcagtggaatagagaacataataaat
<i>xyIR</i> -200-6	attatTTTTatcaatatattttgttaaaaactcgagtgctaaagtattcgaaactttcc
<i>xyIR</i> -500-1-1	cctaggtataatactagtcgagttagacataatagtgagtttagagctagaaatagca
<i>xyIR</i> -500-1-2	tatacttaatgtgctgcagttgacagctagctcagtcctaggtataatactagtcgagt
<i>xyIR</i> -500-2	atacaattacttaactaagatgattgatttctatctctagaaaaaagcaccgactcgg
<i>xyIR</i> -500-3	ccgagtcggtgcttttttctagagatagaaatcaatcatcttagttaagtaattgtat
<i>xyIR</i> -500-4	atttattatgttctctattccactgcagcatactttcaataactttttc
<i>xyIR</i> -500-5	gaaaaaagttattgaaagtatgctgcagtggaatagagaacataataaat
<i>xyIR</i> -500-6	ttttatcaatatattttgttaaaaactcgagtatcactaatatttgaactattagctt
P19	ctccaaatgaagaattaaaagaagtatact
P20	atcatgagcatctttgtcattttg
dCas9-1	aaagggagtgctgactcgagatggataagaaatactcaataggcttagctatcggcaca
dCas9-2	aggaaactttgtggaacaattgcatcgacatcataatcactta
dCas9-3	taagtgattatgatgtcgatgcaattgtccacaaagtttcct
dCas9-4	acggccagtgcaattccgggtcagtcacctcctagctgactcaaa
dsgRNA- <i>spo0A</i> (824)-1-1	gtcctaggtataatactagttttaattgcagatgataatagtttagagctagaaatagc
dsgRNA- <i>spo0A</i> (824)-1-2	agcttggtgcagttgacagctagctcagtcctaggtataatactagtt
dsgRNA- <i>spo0A</i> (824)-2	tttagaaaattatttatattttatagcgccgcgcaaaaaagcaccgactcggtgccact
dsgRNA- <i>spo0A</i> (8052)-1-1	gtcctaggtataatactagttataataaaagaatggacaaaagtttagagctagaaatagc
dsgRNA- <i>spo0A</i> (8052)-1-2	agcttggtgcagttgacagctagctcagtcctaggtataatactagtt
dsgRNA- <i>spo0A</i> (8052)-2	tttagaaaattatttatattttatagcgccgcgcaaaaaagcaccgactcggtgccact
<i>cac2679</i> -1	tctcggtataacccatgttc
<i>cac2679</i> -2	tgtagagtaagctccttctg
<i>cac2679</i> -3	tggaatcacctatacaagag
<i>cac2679</i> -4	caggttcgtttgaagtaatg
<i>cac2071</i> -1	cggactactatgtagttaaac
<i>cac2071</i> -2	ttgcaattgtacctgcaaag
<i>cac2071</i> -3	gcaggtacaattgcaaattgatg
<i>cac2071</i> -4	tgctggaacacctatttgatg
<i>cbei0001</i> -1	gactagaaatgttgcttacc
<i>cbei0001</i> -2	tcttctccaaattcttctc
<i>cbei0001</i> -3	aggaaagcatgtgactattg
<i>cbei0001</i> -4	acatttctagtcgcctttg
<i>cbei1712</i> -1	caacaggcaataactcttgg
<i>cbei1712</i> -2	ctgctgcagagctattaaac
<i>cbei1712</i> -3	gccacatttagatggattag
<i>cbei1712</i> -4	aagagttattgcctgttgag

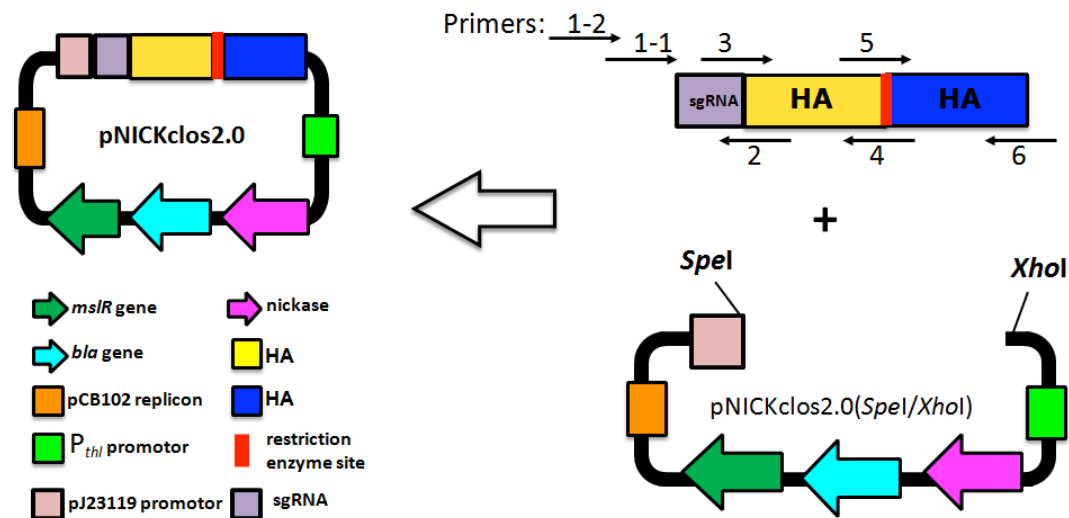
Sequences in bold represent the guide sequence of sgRNA used in genes editing and regulation plasmids construction. The underlined sequence represents the restriction sites. The primers containing “seq” were used to do DNA sequencing to verify the precise insertion of restriction endonuclease sites.

**Supporting information, Table S3: Comparison of genetic editing tools in solventogenic clostridia**

Tools	Time	Frequency	Scarless	Markerless	Insertion	Reference
<b>GroupII intron</b>	3-7days	25%-100% <sup>[2]</sup> 25%-62% <sup>[3]</sup>	No	Yes	Yes	<sup>[2-4]</sup>
<b><i>I-SceI</i></b>	1-2 weeks	3.8%-20%	Yes	Yes	ND	<sup>[1]</sup>
<b><i>mazF</i></b>	1-2 weeks	ND	No	No	Yes	<sup>[5]</sup>
<b><i>upp</i></b>	ND	ND	No	No	Yes	<sup>[6]</sup>
<b><i>pyrE</i></b>	1-2 weeks	14.3%-100%	Yes	Yes	Yes	<sup>[7]</sup>
<b>CRISPR-Cas9 nickase</b>	3 days	6.7%-100%	Yes	Yes	Yes	This study

ND, not determined.

**Supporting information, Figure. S1: Flow chart of construction for pNICKclos2.0 series of plasmids.**



1-2, 1-1, 2, 3, 4, 5, 6 are primers used in the construction of pNICKclos2.0 series of plasmids. HA: Homology arm.

Step 1: Primers 1-1/2 were first used to PCR amplify a sgRNA cassette which was then used as the template with primers 1-2/2 to produce the overlapping extensions at the ends of the sgRNA cassette.

Step 2: Primers 3/4, 5/6 were used to PCR amplify the two homology arms (HAs);

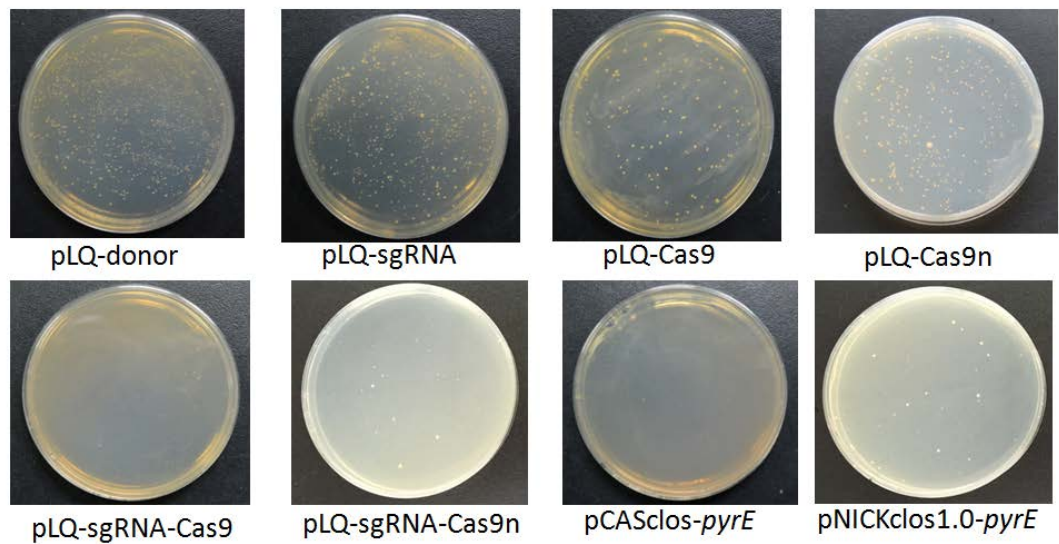
Step 3: The three fragments (sgRNA cassette, two HAs) were then joined by overlap PCR extension using primers 1-2/6;

Step 4: Plasmid pNICKclos2.0 (e.g., pNICKclos2.0-*xyIR*) was digested by *SpeI* and *XhoI*;

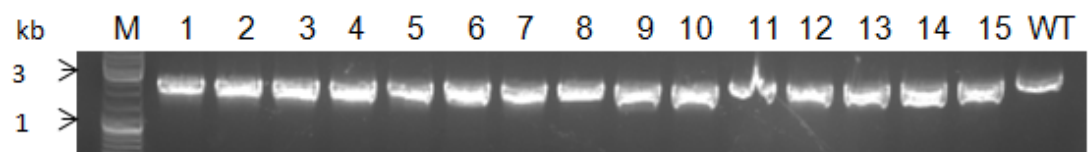
Step 5: The PCR products in Step 3 were fused with the linearized plasmid pNICKclos2.0 in

Step 4 to generate the pNICKclos2.0 series of plasmids

**Supporting information, Figure. S2: Transformation efficiency of different plasmids involved in CRISPR-Cas9-mediated gene editing.**



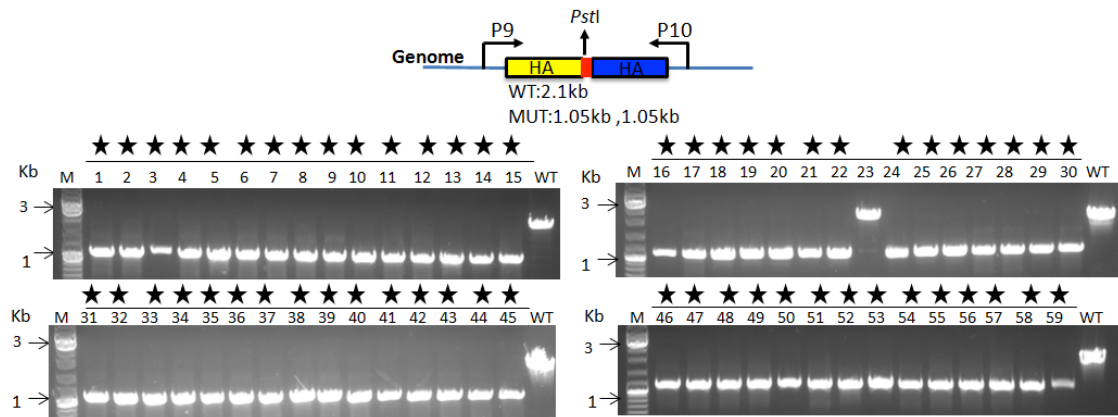
**Supporting information, Figure. S3: Confirmation of transformants of the control plasmid pLQ-Donor.**



*EcoRV* digestion of P1/P2 PCR products was employed to confirm that there were no mutations in the transformants of the control plasmid pLQ-Donor.

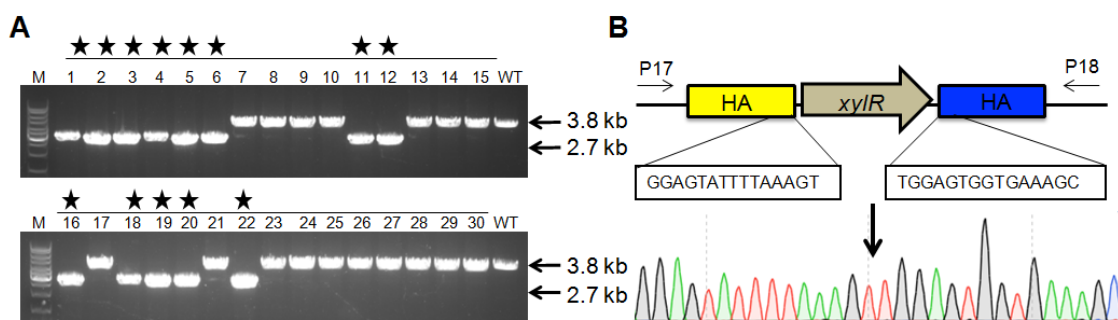


**Supporting information, Figure. S4: The deletion of *xylR* gene was provided as the representative of reliable probability of successful knockouts.**



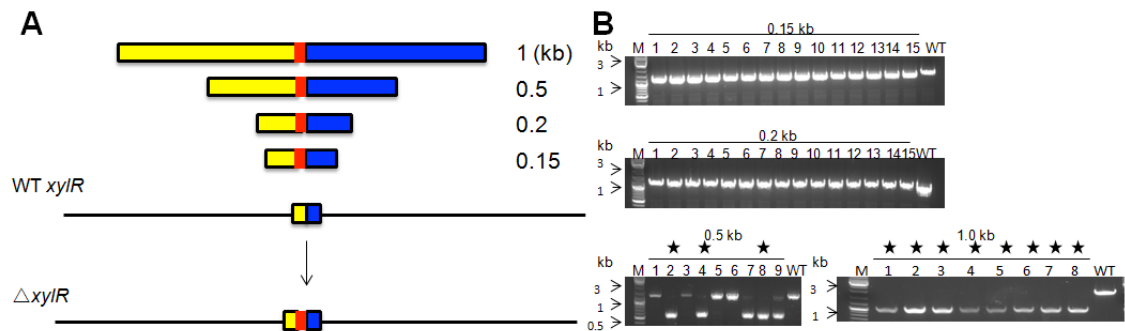
*xylR*-deletion mutants was confirmed by the *Pst*I digestion of P9/P10 PCR products. The genomic DNA of wild-type (WT) *C. beijerinckii* NCIMB 8052 was used as the control. The asterisks indicates the correct mutants. HA, homology arm; WT: wild type; MUT: mutant.

**Supporting information, Figure. S5: Identification of the removal of a larger fragment (1149bp) via pNICKclos2.0 system.**



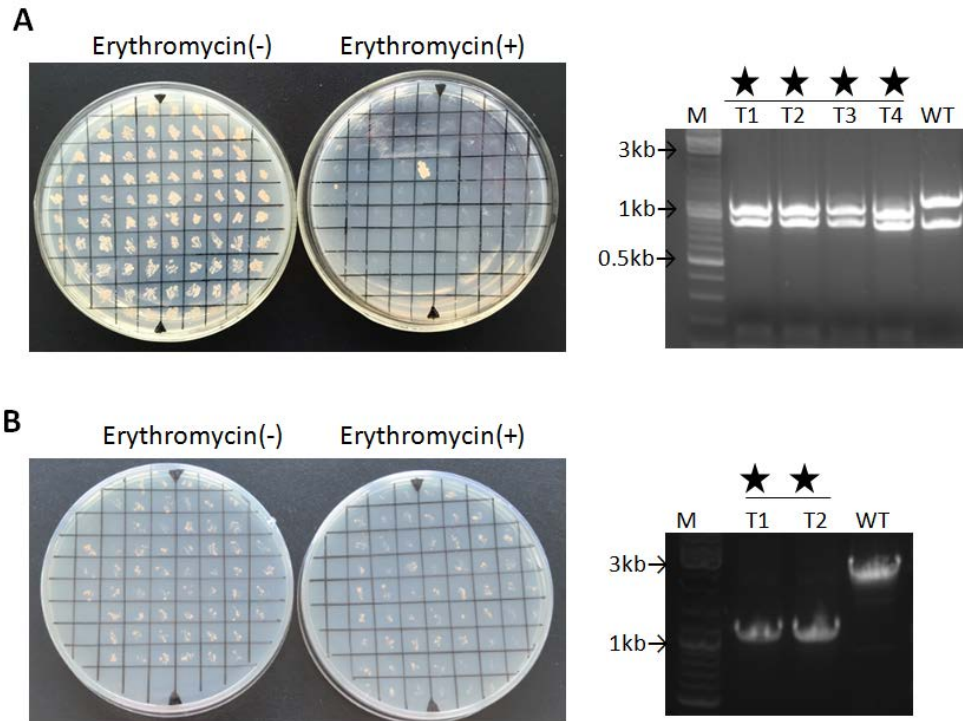
(A) Verification of the complete removal of *xyIR* gene. The positive transformants yielded 2.7 kb amplicons, while the wild type (WT) generated 3.8 kb amplicons. The genomic DNA of wild-type *C. beijerinckii* NCIMB 8052 was used as the control. The asterisks indicates the correct mutants. (B) Sequencing for the mutants harboring the Open Reading Frame-deletion of *xyIR* gene. HA, homologous arms flanking the Open Reading Frame of the *xyIR* gene. P17 and P18 were the primers outside of HA used for PCR identification.

**Supporting information, Figure. S6: Different sizes of homology arms affect the editing efficiency.**



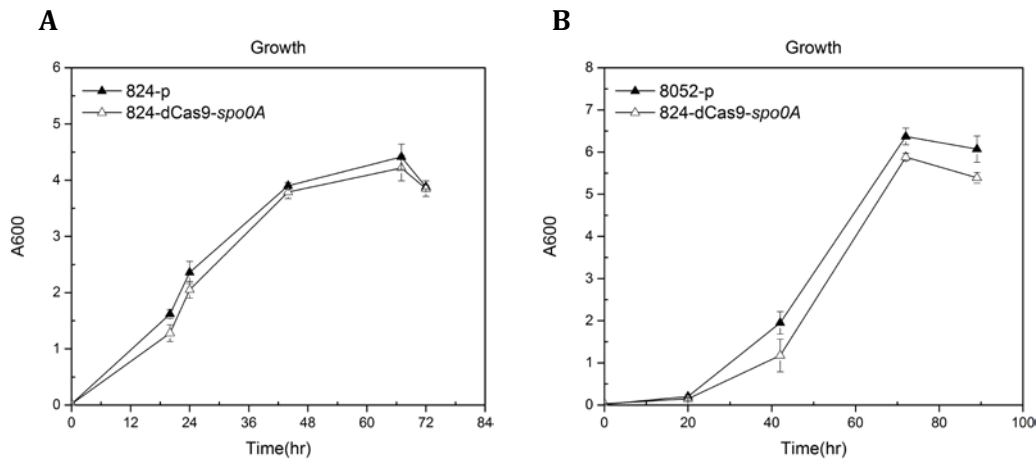
(A) Different sizes of homology arms were designed to edit the *xylR* gene, *PstI* site (red) added between the two homology arms (yellow and blue) was introduced into the target site. (B) Editing efficiency confirmed by *PstI* digestion of PCR products. WT: wild type; the asterisks indicates the correct mutants.

**Supporting information, Figure. S7: Plasmid curing and reconfirm the genotype of the host.**



(A) The clearance of plasmid pNICKclos2.0-*araR* and reconfirm the genotype of *C. beijerinckii* NCIMB 8052 $\Delta$ *araR* during the process of cell transferring. (B) The elimination of plasmid pNICKclo1.0-*pyrE* and reconfirm the genotype of *C. acetobutylicum* ATCC 8241 $\Delta$ *pyrE* under the process of cell transferring. T1-T4 represent the cell in the first round to fourth round culture; the asterisks indicates the correct mutants.

**Supporting information, Figure. S8: The growth of the CRISPR-dCas9-mediated repression strain and the wild type strain.**



(A) Growth of 824-p and 824-dCas9-spo0A strains in P2 batch fermentation. (B) Growth of 8052-p and 8052-dCas9-spo0A strains in XHP2 batch fermentation. Fermentations were performed in triplicate.

**Supporting information, Figure. S9: Sporulation ability of strain 824-dCas9-spo0A and 824-p**



Strain 824-dCas9-spo0A and 824-p were grown in liquid CGM medium containing erythromycin until  $OD_{600}$  reached 0.6. Same concentration cells were spread on CBM plates. Colonies were collected and suspended with 5mL CGM at the 6<sup>th</sup> day, heat shock for 10 min at 80 °C. Cells were manipulated at the same concentration strains of 824-dCas9-spo0A and 824-p. Then, cells were diluted properly and spread on CGM agar.

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