TABLE S1 Strains, plasmids, and oligonucleotide primers used in this study

Strains	**Description**	**Sources**
C. beijerinckii		
NCIMB 8052	Wild type	Lab stock
pta::int(17)	Group II intron inserted at 17/18a bp of *pta*[a]	This study
buk::int(532)	Group II intron inserted at 532/533s bp of *buk*[b]	This study
E. coli		
Turbo	High efficiency chemically competent cell	NEB[c]
Top10	Electrocomp™ competent cell	Invitrogen[d]

Plasmids	**Description & Relevant characteristics**	**References**
pTJ1	Ap^r, Em^r, *C. beijerinckii-E. coli* shuttle vector[e]	(1)
pJIR750ai	L1.LtrB-ΔORF intron and ltrA , targeting *C.perfringens* alpha toxin gene	(2)
pCR-XL-TOPO®	one-step cloning vector for long PCR products	Invitrogen[d]
pYW1	pTJ1 integrated with intron+ltrA, and *ptb* promoter	This study
pYW1-*pta*	pYW1 with intron targeting *pta*	This study
pYW1-*buk*	pYW1 with intron targeting *buk*	This study

Primers	**Sequence (5'-3')**	**Description**
Intron-F	*CCCGGG*ATAATTATCCTTACCAGCCCATAGG	XmaI
Intron-R	CTTGTAGATATGACGACAGGAAGAGT	
ptb-p-F	GTATATC*AAGCTT*GAAGATATATTATATTACGTTC GTGTTGTGA	HindIII
ptb-p-R	GTATATC*ACTAGT*AATCAATGCTATGAATATTTCT TTATACCTT	SpeI
pta-17/18a-IBS	AAAACCCGGGATAATTATCCTTAGCAGTCCATA TTGTGCGCCCAGATAGGGTG	XmaI
pta-17/18a-EBS1d	CAGAT*TGTACA*AATGTGGTGATAACAGATAAGT CCATATTTTTAACTTACCTTTCTTTGT	BsrGI
pta-17/18a-EBS2	TGAACGCAAGTTTCTAATTTCGATTACTGCTCG ATAGAGGAAAGTGTCT	
EBS universal	CGAAATTAGAAACTTGCGTTCAGTAAAC	Universal primer for knockout re-targeting
pta-17/18a-F	TTGTTATTTTACAGGAGTATTGTCTGA	Confirmation of knockout for *pta*
pta-17/18a-R	TCTTTCCTCGTTTCCCTCTG	Confirmation of knockout for *pta*
buk-532/533s-IBS	AAAA*CCCGGG*ATAATTATCCTTAATGAACACCT AAGTGCGCCCAGATAGGGTG	XmaI
buk-532/533s-EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGT CACCTAAATTAACTTACCTTTCTTTGT	BsrGI
buk-532/533s-EBS2	TGAACGCAAGTTTCTAATTTCGGTTTTCATCCG ATAGAGGAAAGTGTCT	
buk-532/533s-F	GCTTCTAACCTTGGCGGAAT	Confirmation of knockout for *buk*
buk-532/533s-R	AACGCCTCCACCCATATGTA	Confirmation of

		knockout for *buk*
pYW1-cure-F	GCTTTCGTTTCGTTCCCATA	Detection of plasmid curing
pYW1-cure-R	TGAATTGATTGGGCCCTCTA	Detection of plasmid curing
Intron-SB-F	CCTATGGGAACGAAACGAAA	Amplification of Southern blotting probe
Intron-SB-R	CGAGTACTCCGTACCCTTGC	Amplification of Southern blotting probe
buk-qPCR-U-F	TGTTGGTAGAGGTGGAATGCT	q-RT-PCR, upstream of intron insertion
buk-qPCR-U-R	GCATGAGGTCCTTGAACTCC	q-RT-PCR, upstream of intron insertion
buk-qPCR-D-F	GGAAAAGGTGGATTTGTTGGT	q-RT-PCR, downstream of intron insertion
buk-qPCR-D-R	ACTGGGGCTATCCATTCAACT	q-RT-PCR, downstream of intron insertion
bukII-qPCR-F	TAGTTGGAAGAGGTGGCCTTT	q-RT-PCR, for *bukII*
bukII-qPCR-R	CTGGCACTCCTGAAAGTCTTG	q-RT-PCR, for *bukII*
bukIII-qPCR-F	CACAAACGATGCTAGGGTTGTA	q-RT-PCR, for *bukIII*
bukIII-qPCR-R	AGCATTTCATCTTCACCTGGAT	q-RT-PCR, for *bukII*
16s-qPCR-F	CCGCTAACGCATTAAGTATTCC	q-RT-PCR, for 16s RNA as endogenous control
16s-qPCR-R	CTTAACCCAACATCTCACGACA	q-RT-PCR, for 16s RNA as endogenous control

[a] 17/18a, between 17 and 18 bp in the antisense direction

[b] 532/533s, between 532 and 533 bp in the sense direction

[c] New England Biolabs Inc., Ipswich, MA

[d] Invitrogen Inc., Grand Island, NY

[e] Ap, ampicillin; Em, erythromycin

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1. Jesse TW. 2003. Genetic characterization and manipulation of solvent-producing clostridia. PhD dissertation. University of Illinois at Urbana-Champaign, Urbana, IL.
2. Chen Y, McClane BA, Fisher DJ, Rood JI, Gupta P. 2005. Construction of an alpha toxin gene knockout mutant of *Clostridium perfringens* type A by use of a mobile group II intron. Appl. Environ. Microbiol. 71:7542-7547.

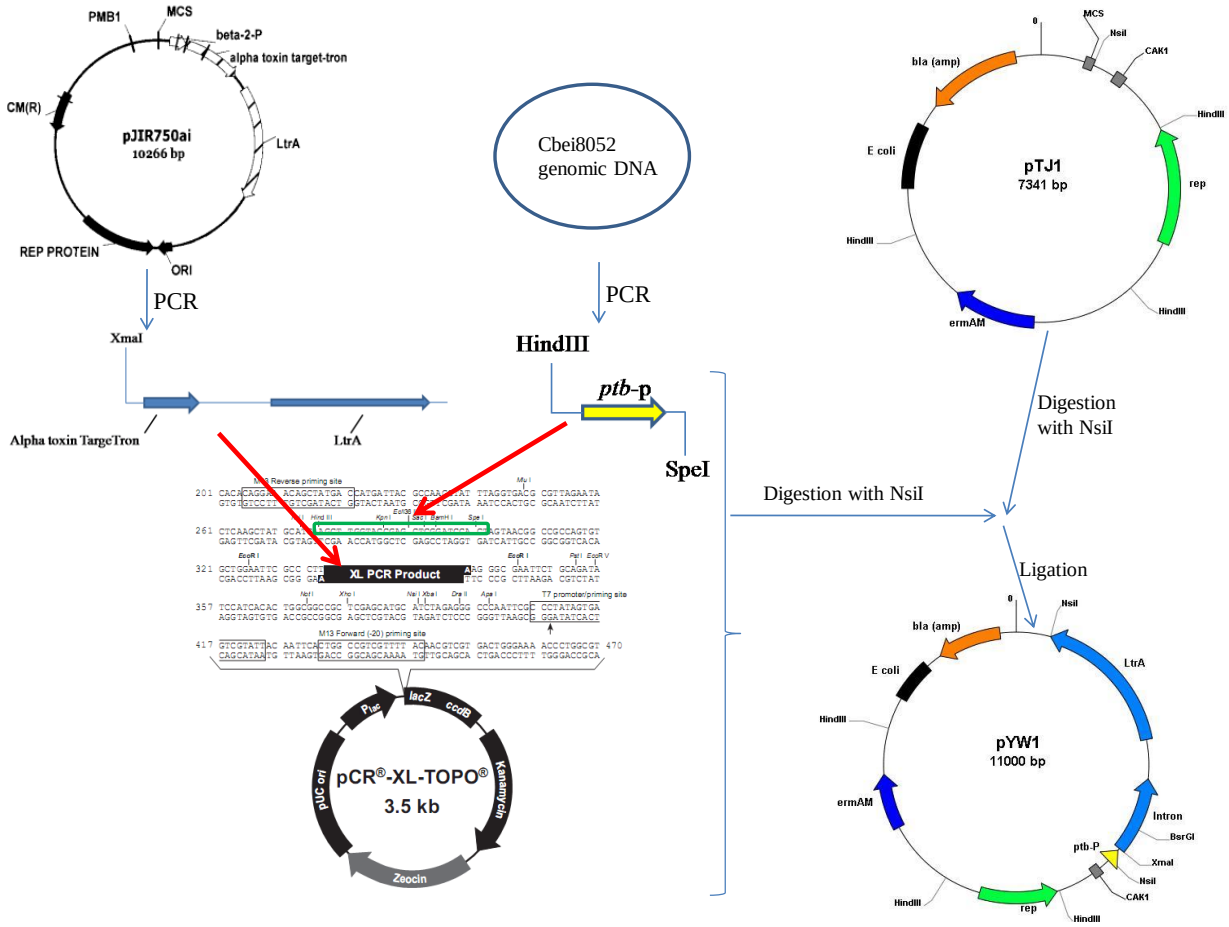

FIG S1 The schematic diagram for construction of the Targetron plasmid pYW1 for gene knockout in *C. beijerinckii*. The DNA fragment containing the LtrB intron and LtrA gene in pJIR750ai (1) was first amplified by PCR, with an *Xma*I restriction enzyme site introduced for later intron re-targeting purposes. The PCR product was cloned into the cloning site of pCR-XL-TOPO® vector (Invitrogen, Grand Island, NY). The *ptb* promoter from *C. beijerinckii* 8052 genomic DNA was amplified by PCR with introduction of the *Hind*III and *Spe*I sites into the fragment. The PCR product was integrated into the *Hind*III and *Spe*I sites of the pCR-XL-TOPO® vector containing the intron fragment. The resulting cloning vector was digested with *Nsi*I, and ligated into pTJ1 digested with the same enzyme. The plasmid with the insertion at the desired direction was verified by sequencing and named pYW1 for later intron re-targeting and gene disruption.

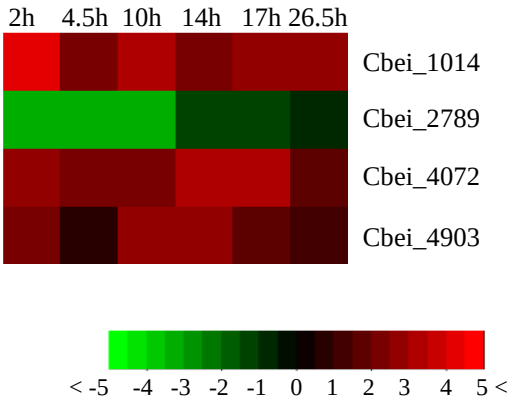

FIG S2 The dynamic transcriptional profiles of lactate dehydrogenase genes over the batch fermentation process based on the RNA-Seq data published earlier (2).

REFERENCES

1. Chen Y, McClane BA, Fisher DJ, Rood JI, Gupta P. 2005. Construction of an alpha toxin gene knockout mutant of *Clostridium perfringens* type A by use of a mobile group II intron. Appl. Environ. Microbiol. 71:7542-7547.
2. Wang Y, Li X, Mao Y, Blaschek H. 2012. Genome-wide dynamic transcriptional profiling in *Clostridium beijerinckii* NCIMB 8052 using single-nucleotide resolution RNA-Seq. BMC Genomics 13:102.