Strain or plasmid		Description	Source or reference
Propionibacterium acidipropionici 1.2232	CGMCC	Wild type	China General Microbiological Culture Collection Center
$\Delta otsA$		$\Delta otsA::\Omega$ : Wild type with insertion of $\Omega$ cassette and deletion in the <i>otsA</i> ORF	This work
$\Delta treY$		$\Delta tre Y:: \Omega$ : Wild type with insertion of $\Omega$ cassette and deletion in the tre Y ORF	This work
$\Delta ots A \Delta treY$		$\Delta otsA::\Omega/\Delta treY$ : Wild type with deletion in the <i>otsA</i> and <i>trey</i> ORFs and insertion of $\Omega$ cassette in the <i>otsA</i> ORF	This work
E. coli			
DH5a		supE44 $\Delta$ lacU169 ( $\Phi$ 80 lacZ $\Delta$ M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 $\lambda$ -	Vazyme Biotech (Nanjing) Co., Ltd.
JM109		endA1 recA1 gyrA96 hsdR17 supE44 $\lambda$ <sup>-</sup> $\Delta$ (lac-proAB)/F' [ tra $\Delta$ 36 proAB <sup>+</sup> lacZ $\Delta$ M15]	Vazyme Biotech (Nanjing) Co., Ltd.
JM109 (λ <i>pir</i> )		JM109 lysogenized with $\lambda$ pir bacteriophage	Vazyme Biotech (Nanjing) Co., Ltd.
S17-1		Tp <sup>r</sup> Sm <sup>r</sup> hsdR pro recA RP4-2-Tc::Mu-Km::Tn 7 in chromosome	Vazyme Biotech (Nanjing) Co., Ltd.
S17-1 (λ <i>pir</i> )		S17-1 lysogenized with $\lambda pir$ bacteriophage	Vazyme Biotech (Nanjing) Co., Ltd.
Plasmid			
pBluescript II SK+		Ap <sup>r</sup> ; cloning vector	Stratagene
pTK106		Ap <sup>r</sup> Sm <sup>r</sup> /Spc <sup>r</sup> ; source of the Sm <sup>r</sup> /Spc <sup>r</sup> gene ( $\Omega$ cassette)	Prentki and Krisch (1984)
pJP5603		Km <sup>r</sup> ; R6K-based suicide vector	Kai M. Thormann et al. (2004)
pHSGSKm		Cm <sup>r</sup> Km <sup>r</sup> ; pHSG396 carrying the Km <sup>r</sup> gene and <i>sacRsacB</i>	Masuda et al. (2002)
pJPmobsac		Km <sup>r</sup> ; R6K-based suicide vector carrying <i>sacRsacB</i>	This work
pBOTSA I		pBII with PCR fragment containing upstream of otsA	This work
pBOTSA II		pBII with PCR fragments containing upstream and downstream of <i>otsA</i>	This work

## Table S1. Bacterial strains and plasmids used in this work

pBOTSAX	pBOTSA II with $\Omega$ cassette insertion between PCR fragments	This work
pJPOTSA	pJP5603 carrying PCR fragments of $otsA$ with $\Omega$ cassette insertion	This work
pBTREY I	pBII with PCR fragment containing upstream of <i>treY</i>	This work
pBTREY II	pBII with PCR fragments containing upstream and downstream of <i>treY</i>	This work
pBTREYX	pBOTSA II with $\Omega$ cassette insertion between PCR fragments	This work
pJPTREY	pJP5603 carrying PCR fragments of <i>treY</i> with $\Omega$ cassette insertion	This work
pJPmobsacTREY	pJPmobsac carrying PCR fragments of treY	This work