To construct the standard plasmid for absolute quantification of CLas in the samples, a fragment of 16S rRNA were amplified using primers HLBas-R and HLBas-F. The PCR mixture used was: 50 ng of total DNA from CLas-positive budwood, 10 pmol of upstream and downstream primer, 10 μL of high-fidelity DNA polymerase/dNTP master mix, H_2O to a total volume of 20 μL. The amplicons were ligated to the pEASY-blunt vector (Transgen, Beijing, China) referring to the cloning kit. The recombinant plasmid was transformed into Escherichia coli Trans-T1 by heat shock method. The transformed strains were screened by the pressure of 50 mg/L kanamycin. Single colonies were picked for culture. Plasmid were extracted using a plasmid mini-kit from Shanghai Sangon Bioengineering Co., Ltd. Plasmids were sequenced.

Plasmid information:

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DEFINITION  synthetic circular DNA
ACCESSION   .
VERSION     .
KEYWORDS    .
SOURCE      synthetic DNA construct
  ORGANISM  synthetic DNA construct
REFERENCE   1  (bases 1 to 4267)
  AUTHORS   CCQQ
  TITLE     Direct Submission
  JOURNAL   Exported Jun 13, 2022 from SnapGene 4.2.4
            http://www.snapgene.com
FEATURES             Location/Qualifiers
     source          1..4267
                     /organism="synthetic DNA construct"
                     /mol_type="other DNA"
     CDS             1..213
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                     /gene="lacZ fragment"
                     /product="LacZ-alpha fragment of beta-galactosidase"
                     /label=lacZ-alpha

/translation="KGQFAAAKFNSPYSESYYNSLAVVLQRRDWENPGVTQLNRLAAHP
                     PFASWRNSEEARTDRPSQQLRSLYVR"
     promoter        complement(35..53)
                     /label=T7 promoter
                     /note="promoter for bacteriophage T7 RNA polymerase"
     primer_bind     complement(60..76)
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                     /note="common sequencing primer, one of multiple similar
                     variants"
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CDS 214..516
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/label=ccdB
/note="Plasmids containing the ccdB gene cannot be
propagated in standard E. coli strains."
/translation="QFKVYTYKRESRYRLFVDVQSDIIDTPGRRMVIPLASARLLSDKV

SRELYPVVHIGDESWRMMTTDMASVPVSVIGEEVADLSHRENDIKNAINLMFWGI"
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/gene="aph(3')-II (or nptII)"
/product="aminoglycoside phosphotransferase from Tn5"
/label=NeoR/KanR
/note="confers resistance to neomycin, kanamycin, and G418
(Geneticin(R))"

/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRP

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GLAPAELFARLKASMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRYQDIA
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related antibiotics"

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