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Highly reliable polymerase chain reaction assay for citrus huanglongbing (citrus greening disease) using the sucrose synthase gene as an internal control

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ABSTRACT

The polymerase chain reaction (PCR) was used with an internal control to exclude false-negative reactions in a citrus huanglongbing (HLB) assay. Degenerated primers, CSSF-1 (5'-GACACTGTTGGWCAGTATGA-3') and CSSR-1 (5'-TCRTACAVTGCAGGTTGCAC-3'), for the internal control were constructed based on the sucrose synthase genes of *Citrus unshiu*, *Medicago sativa*, *Daucus carota*, *Arabidopsis thaliana* and *Phaseolus vulgaris*. Multiplex PCR with the CSS primer and the Ol1-Ol2c primer amplified two DNA fragments corresponding to the sucrose synthase gene and the '*Candidatus* Liberibacter asiaticus' 16S rDNA gene from '*Candidatus* L. asiaticus' infected citrus DNA, respectively. A DNA fragment of the sucrose synthase gene was solely amplified from healthy samples. No amplified fragment was detected in incomplete reactions.

Key words: citrus huanglongbing, PCR assay, internal control, sucrose synthase gene

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