

附件3、澳大利亞農業部指定之ToBRFV引子對及PCR檢測方法

Approved PCR testing protocol and primers for ToBRFV

Date	approved Primers	PCR protocol
1 April 2019	F-5476 – GAAGAAGTTGTTGATGAGTTCAT R-6287 – GATTTAAGTGGAGGGAAAAACAC Reference Levitzky, N, Smith, E, Lachman, O, Luria, N, Mizrahi, Y, Bakelman, H, Sela, N, Laskar, O, Milrot, E & Dombrovsky, A, 2019, 'The bumblebee <i>Bombus terrestris</i> carries a primary inoculum of <i>Tomato brown rugose fruit virus</i> contributing to disease spread in tomatoes' <i>PLoS ONE</i> , vol. 14, no. 1, p.e0210871.	Protocol as specified by Levitzky et al. 2019
1 April 2019	ToBRFV - F - AATGTCCATGTTTGTACGCC ToBRFV - R - CGAATGTGATTTAAACTGTGAAT Reference Alkowni, A, Alabdallah, O & Fadda, Z 2019, 'Molecular identification of <i>tomato brown rugose fruit virus</i> in tomato in Palestine' <i>Journal of Plant Pathology</i> , available at DOI 10.1007/s42161-019-00240-7.	Protocol as specified by Alkowni
7 June 2019	CSP1325 – F – CATTGAAAGTGCATCCGGTTT CSP1325 – R – GTACCACGTGTGTTTGCAGACA CSP1325 – P – ([VIC*] – ATGGTCCTCTGCACCTGCATCTTGAGA [BHQ1]) *can be FAM Reference ISHI-Veg 2019. Detection of Infectious <i>Tomato brown rugose fruit virus</i> (ToBRFV) in Tomato and Pepper Seed. https://www.worldseed.org/wp-content/uploads/2019/05/Tomato-ToBRFV_2019_1.2.pdf	Australian protocol (RTqPCR) Set up RT-PCR cycles: ● 48 °C for 30 min ● 94 °C for 5 min ● 40 cycles of 94 °C for 10 sec., 60 °C for 30 sec.
24 June 2019	CaTa28 – F – GGTGGTGTCTAGTGTCTGTTT CaTa28 – R – GCGTCCTGGTAGTGATGTT CaTa28 – P – (6FAM – AGAGAATGGAGAGAGCGGACGAGG [BHQ1]) Reference ISHI-Veg 2019. Detection of Infectious <i>Tomato brown rugose fruit virus</i> (ToBRFV) in Tomato and Pepper Seed. https://www.worldseed.org/wp-content/uploads/2019/05/Tomato-ToBRFV_2019_1.2.pdf	Australian protocol (RTqPCR) Set up RT-PCR cycles: ● 50 °C for 10 min ● 95 °C for 3 min ● 40 cycles of 95 °C for 10 sec., 60 °C for 60 sec.

- Note, PCR-amplified products from positive test results can be sequenced to confirm detection of ToMMV. The outcome of the identified sequence results must be provided to the department.