



**Figure S1.** Comparison of incubation conditions in the SPEX method for detecting cucumber mosaic virus (CMV) in *N. benthamiana*. CMV cDNA was detected simultaneously with the cDNA of *ndhB* mRNA by RT-PCR using Go-to DNA polymerase from nucleic acids extracted from leaves using the standard PEX (lane Std), and SPEX-A, SPEX-B, and SPEX-C (lanes A–C) methods. Duplex RT-PCR products were electrophoresed in a 2% agarose gel. Lanes, NTC: no template control; M: molecular size marker Gene Ladder 100.

**Table S1. List of primers used in PCR**

Target	Primer name	Primer sequence 5'-3'	Annealing temp. (°C)	Size of PCR product (bp)	Ref.
chrysanthemum stunt viroid (CSVd)	CSV-1P CSV-1M	CTTAGGACCCCACTCCTGCG CCGCGATCTCGTCGGACTTC	61	348	[11,35]
citrus exocortis viroid (CEVd)	PCEV-1P PCEV-1M	GCTCCACATCCGATCGTC TGGACGCCAGTGATCCGC	50	332	[11]
potato virus Y (PVY) <i>coat protein</i>	PVYCP6P PVYCP6M	CGTCCAAAATGAGAATGCC TCTTGTTACTGATGCCAC	55	577	[37]
cucumber mosaic virus (CMV) <i>2a</i>	CM2a-1P CM2a-1M	TTCCAGAGATGCCTTCGAGAACG TCCATCACCTTAGCTTCCATGTTG	55	470	This study
apple fruit crinkle viroid (AFCVd)	AFCV-5P AFCV-5M	GCCCTGGGCTCCAAC TAGTGG ACTGGTTGGGACCGCTGGGAC	55	308	This study
hop latent viroid (HLVd)	HLVd-1P HLVd-1M	GGATACAAC TCTTGAGCGCC TAGTTTCCAAC TCCGGCTGG	50	250	[16]
hop latent virus (HpLV) <i>replicase</i>	HLV-5P HLV-9M	GCAAAAGCAGCGCAGAGTATAG TCGCCTGAGAAATGCATTATAGC	50	359	[15]
<i>ndhB</i> <sup>a</sup> mRNA	AtropaNad2.1a AtropaNad2.2b	GGACTCCTGACGTATACGAAGGATC AGCAATGAGATTCCCCAATATCAT	50–61	188	[12,13]

<sup>a</sup> NADH dehydrogenase subunit 2 gene