**Supplementary Table 1.** Oligonucleotide primers used in reverse-transcription-polymerase chain reaction

| Target | Primer[a] | Sequence[b] | Target size (bp) | References |
|---|---|---|---|---|
| CymMV | CymMVK-F | 5′-ACAATAATTTGAAATAATCATGGGA-3′ | 716 | Chung et al. (2010) |
| | CymMVK-R | 5′-AAAACCACACGCCTTATTAAGTTTG-3′ | | |
| ORSV | ORSVK-F | 5′-ACGCACAATCTGATTCGTATTGAA-3′ | 528 | Chung et al. (2010) |
| | ORSVK-R | 5′-TATCAACGTTATTTTCCTAAATAT-3′ | | |
| CyCMV | CyCMV-F | 5′-TTAATGGCGAAGCGCAAATC-3′ | 792 | Yoon et al. (2018) |
| | CyCMV-R | 5′-TCATCGGTTCAACGAGCTAGC-3′ | | |
| *nad5* gene | mt-F2 | 5′-GCTTCTTGGGGCTTCTTGTTCGATA-3′ | 185 | Lee and Chang (2006) |
| | mt-R1 | 5′-ATCTCCAGTCACCAACATTGGCAT-3′ | | |

## References


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Lee, S.-C. and Chang, Y.-C. 2006. Multiplex RT-PCR detection of two orchid viruses with an internal control of plant *nad5* mRNA. *Plant Pathol. Bull.* 15:187-196.

Yoon, J. Y., Kwon, S. J., Cho, I. S. and Choi, G. S. 2018. First report of cymbidium chlorotic mosaic virus infection in *Cymbidium goeringii* in South Korea. *Plant Dis.* 102:2665.

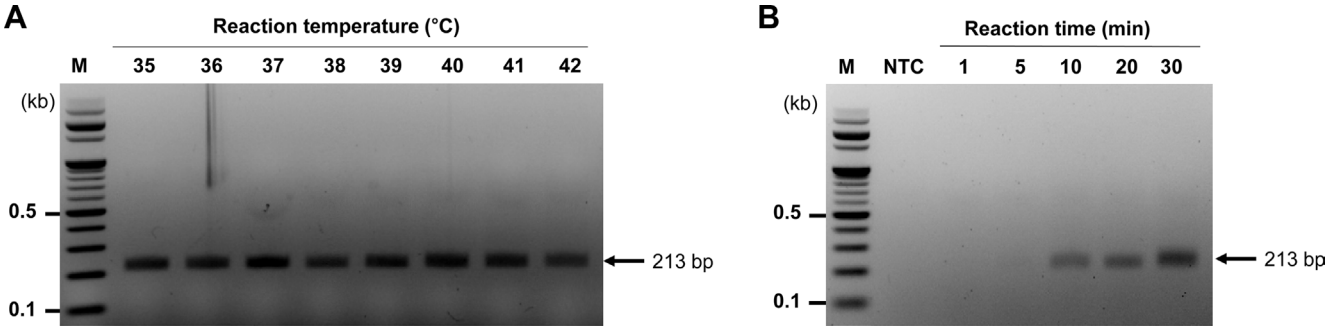

**Supplementary Table 2.** Design of primer sets used for reverse-transcription-recombinase polymerase amplification assay

| Target gene | Primer | Sequence (5′→3′) | Product size (bp) |
|---|---|---|---|
| CP | CymMVCP-1-F | 5′-CTGCCACTTACTCCGCTGCCGACCCCACTTC-3′ | 238 |
| | CymMVCP-1-R | 5′-AGAAACCGAGCAGGGTAGCACTCTTGGACGCC-3′ | |
| | CymMVCP-2-F | 5′-ACTCACCTGTCACCTCCTCCATCGcCACCCC-3′ | 289 |
| | CymMVCP-2-R | 5′-CCAGCATCAGATTCCACACCACTTTTGCGTAGTA-3′ | |
| | CymMVCP-1s-F | 5′-CTGCCACTTACTCCGCTGCCGACC-3′ | 236 |
| | CymMVCP-1s-R | 5′-AGAAACCGAGCAGGGTAGCAC-3′ | |
| | CymMVCP-2s-F | 5′-ACTCACCTGTCACCTCCTCCATCGCC-3′ | 289 |
| | CymMVCP-2s-R | 5′-GCCAGCATCAGATTCCACACCAC-3′ | |
| TGB1 | CymMVT1-1-F | 5′-AGCACCAATCTGCCCCTTTCCTCACCCTTAGT-3′ | 211 |
| | CymMVT1-1-R | 5′-TAATTGTACTCGTCTACGATAGCACCGTCTGGC-3′ | |
| | CymMVT1-2-F | 5′-CTTGACCCTAGCAACCGCATATACCAACAGTG-3′ | 321 |
| | CymMVT1-2-R | 5′-CACTTCCTCGTCAGCAATGGTGAATCCAGAG-3′ | |
| | CymMVT1-3-F | 5′-CTCACCATCTTCGATTTCAATGCCAGGTTTAG-3′ | 213 |
| | CymMVT1-3-R | 5′-TGAGTAGGTGGACAGTAGTTTATCTGCTTAGT-3′ | |
| | CymMVT1-1s-F | 5′-GCACCAATCTGCCCCTTTCCTCAC-3′ | 211 |
| | CymMVT1-1s-R | 5′-TAATTGTACTCGTCTACGATAGCA-3′ | |
| | CymMVT1-2s-F | 5′-CTTGACCCTAGCAACCGCATA-3′ | 321 |
| | CymMVT1-2s-R | 5′-CACTTCCTCGTCAGCAATGGTG-3′ | |
| | CymMVT1-3s-F | 5′-CTCACCATCTTCGATTTCAATGC-3′ | 213 |
| | CymMVT1-3s-R | 5′-TGAGTAGGTGGACAGTAGTTTATCTGC-3′ | |

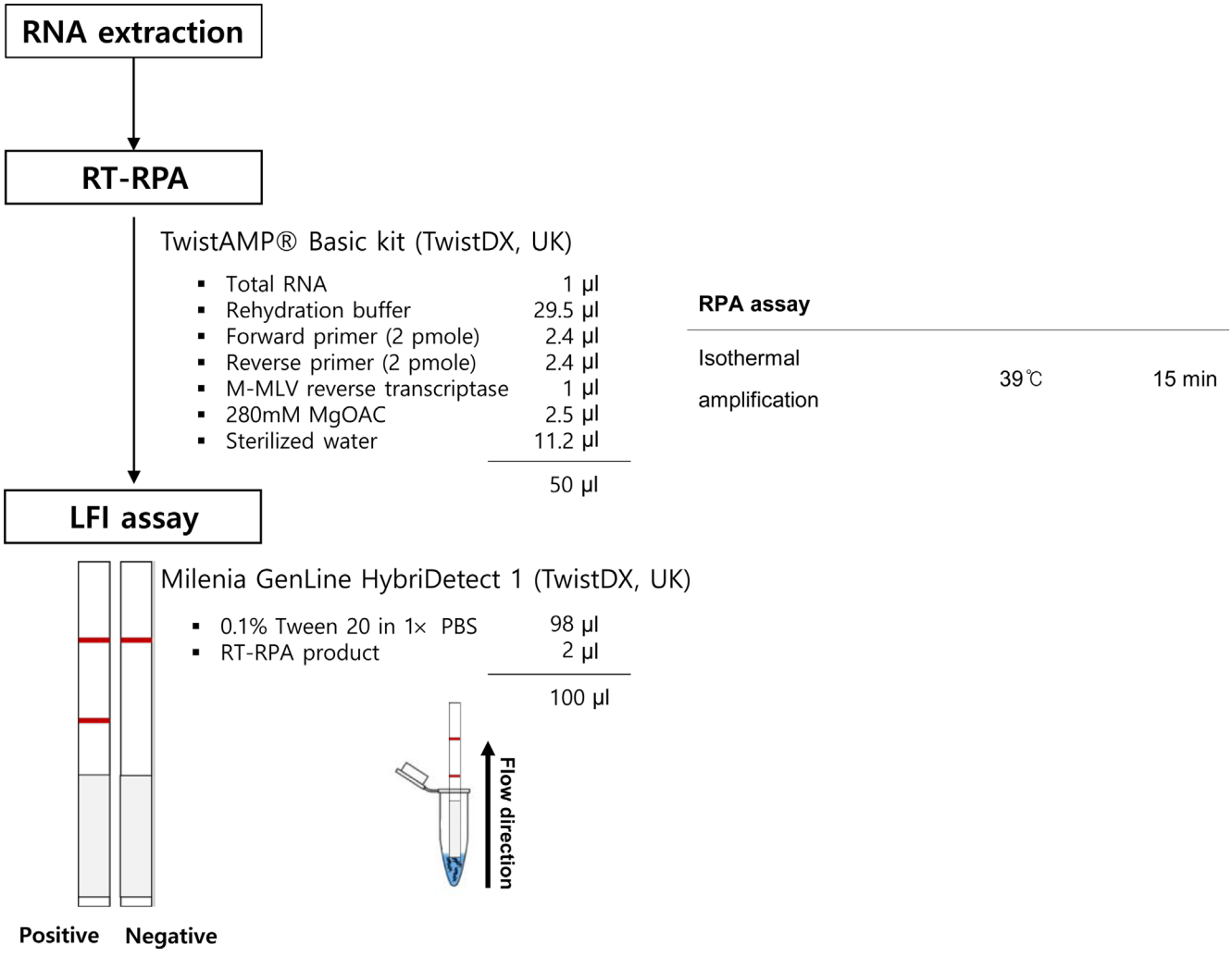
**Supplementary Table 3.** Summarized result of screening of primer set for reverse-transcription-recombinase polymerase amplification assay

| Primer set | 1st exp. | | 2nd exp. | | 3rd exp. | |
|---|---|---|---|---|---|---|
| | NTC[a] | CymMV[b] | NTC | CymMV | NTC | CymMV |
| CymMVCP-1 | –[c] | – | – | – | – | – |
| CymMVCP-2 | – | – | – | – | – | – |
| CymMVCP-1s | – | – | – | – | – | – |
| CymMVCP-2s | – | – | + | – | | – |
| CymMVT-1-1 | – | – | – | – | – | + |
| CymMVT-1-2 | – | – | – | – | – | + |
| CymMVT-1-3 | – | – | – | + | – | + |
| CymMVT-1-1s | – | – | – | – | – | – |
| CymMVT-1-2s | – | – | – | + | – | + |
| CymMVT-1-3s | – | + | – | + | – | + |

[a]NTC(no template control) used water instead RNA as a negative control.
[b]Total RNA at a concentration of 100 ng/μl which was extracted from the cymbidium mosaic virus (CymMV)-infected cymbidium plant was used.
[c]–: negative reaction, +: positive reaction.




**Supplementary Fig. 1.** Establishment of optimal reaction temperature and time of the detection of cymbidium mosaic virus (CymMV) by transcription recombinase polymerase amplification (RT-RPA). (A) Determination of optimal temperature of RT-RPA reaction. The RT-RPA products were visualized from 35°C to 42°C on the 2% agarose gel. (B) Assessment of optimal reaction time of RT-RPA at 39°C of optimal condition. The size of RT-RPA products were approximately 213 bp. Lane M is a 100 bp DNA marker (Enzynomics, Korea).




**Supplementary Fig. 2.** Procedure of reverse transcription recombinase polymerase amplification (RT-RPA) assay combined with a lateral flow immunostrip (RT-RPA-LFI) assay for the detection of cymbidium mosaic virus.