

Supplementary Materials

One-Enzyme RTX-PCR for the Detection of RNA Viruses from Multiple Virus Genera and Crop Plants

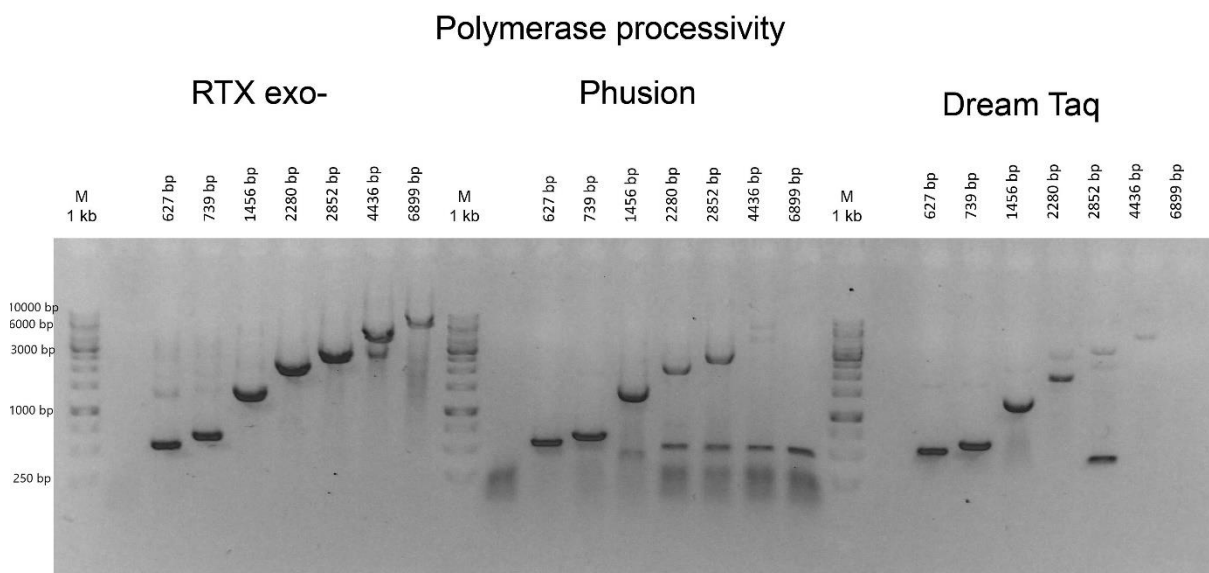


Figure S1. Estimate of processivity of thermostable polymerases. Universal domestication plasmid pUPD2 [56] with cloned inserts of various sizes (from 332 to 6604) were used as a template in PCR with universal sequencing primers pUPD F2/R2 [38]. 30 cycles of PCR program consisting of 30s denaturation at 94°C, 30s annealing at 55°C and short extension for 10s at 72°C were run with 3 different polymerases: RTX exo-, Phusion (ThermoScientific), and DreamTaq (ThermoScientific). Under these settings, the RTXexo- can be used to amplify products up to 5-6 kb in length. The marker is the GeneRuler 1 kb bp Plus DNA ladder from ThermoScientific.

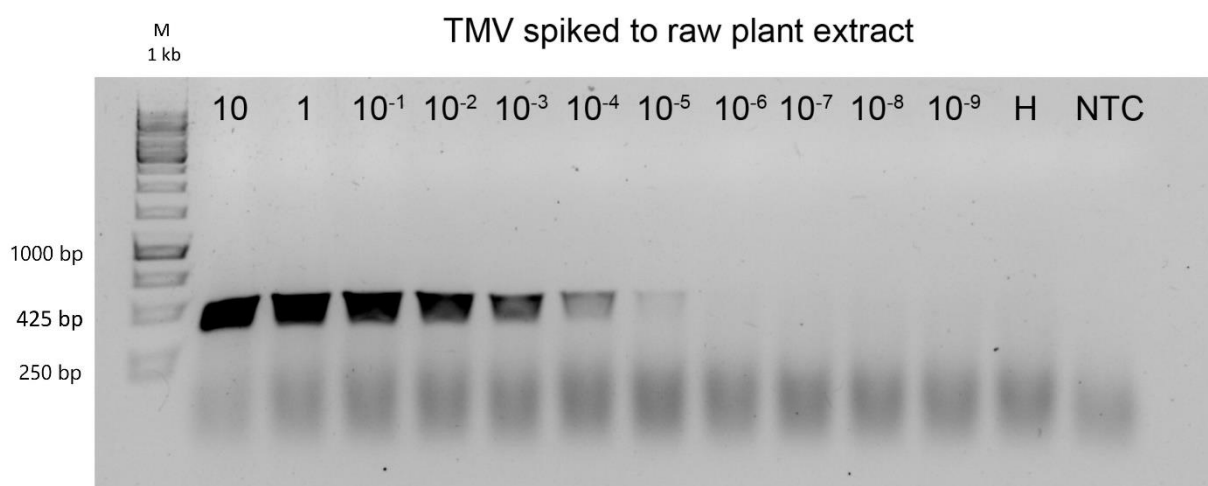


Figure S2. RT-PCR virus detection in crude extracts of *Nicotiana benthamiana*. Purified TMV was added to the extract of uninfected healthy plants to a final concentration of 10 ng/μl, and a 10-fold dilution series was prepared using extracts of healthy plants. 1 μl of the extracts was used as a template for RT-PCR using the RTX enzyme. Numbers indicate ng of pure TMV virus in each

reaction. H= extract from uninfected control plants, NTC = non-template control. The marker is the GeneRuler 1 kb bp Plus DNA ladder from ThermoScientific.

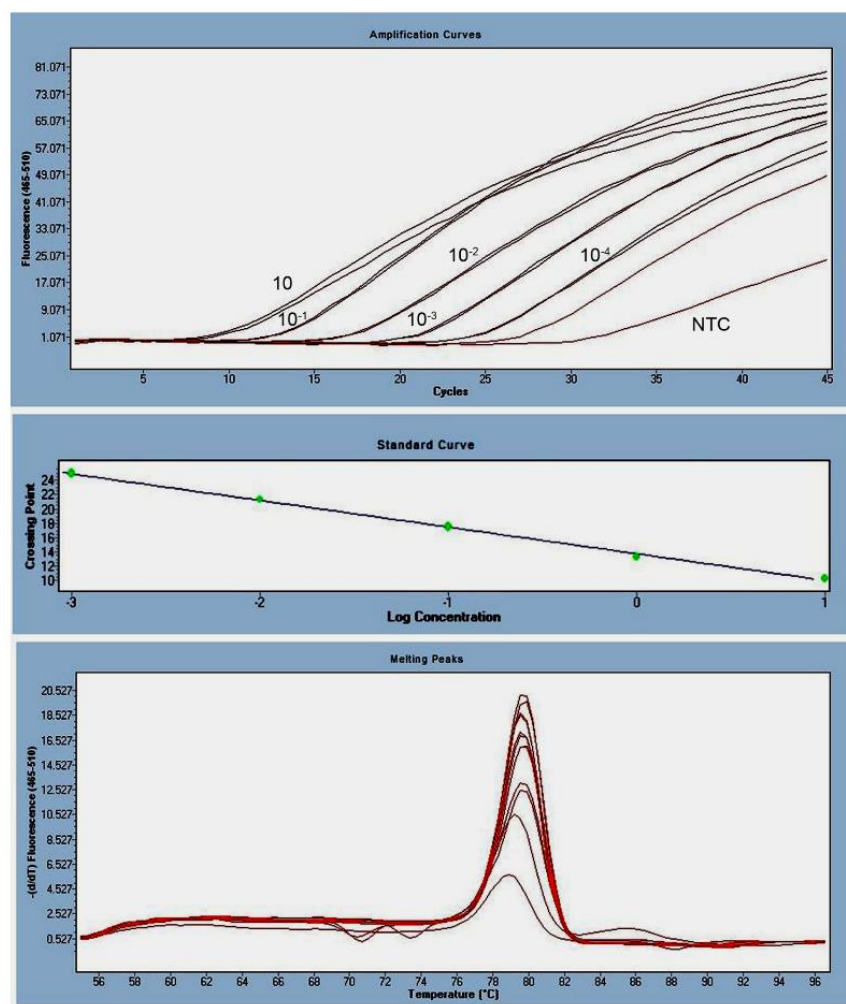


Figure S3. RT-qPCR reactions with the RTX exo- enzyme. Calibration curve constructed with the purified TMV preparation 10-fold serially diluted at 2.5×10 ng to 2.5×10^{-5} ng/reaction. Reactions were run in duplicates. The upper panel shows the real-time fluorescence measurements, the middle panel shows the standard calibration curve, and the lower panel shows the melting curve analysis of amplified fragments. .

Table S1. List of primers used in this work.

| Virus | Genus | Primer Name | Primer sequence (5'-3') | Nucleotide Position | PCR Fragment bp | Reference |
|-----------------------------------|--------------------|----------------|--|---------------------|-----------------|---------------|
| <i>Tobacco mosaic virus (TMV)</i> | <i>Tobamovirus</i> | TMV-MP-detAS | TGCAAGCCTGATCGACATAG | 5507-5526 | | in this study |
| | | TMV-MP-detS | TCTGTTTAGCCGGTTTGGTC | 5102-5121 | 425 | in this study |
| | | Gb2-MP-F | GCGCCGTCTCGCTCGA ATGGCTCTAGTTGTAAAGGAAAA* | 4903-4926 | 624 + 16 | in this study |
| | | TMV-Rep-BsaI-S | GGAGTTCAGAAGATCTCTTTGTGATG | 4740-4752 | 787 | in this study |
| | | TMV-LB | GAAACACTGTGATCATTGCTGC | 4426-4447 | 1101 | in this study |
| | | TMV-rep-4080S | CCGTGTGTTAGTGAGCTTACTAGGC | 4080-4104 | 1447 | in this study |
| | | TMV-rep-3148S | CTCTGCTTTCAAGAGGGTATTCAG | 3148-3171 | 2379 | in this study |
| | | GB-TMV-3_4 F | GCGCCGTCTCGCTCGAATG CTTTCATGATGAATTTGGGAAAAG* | 2728-2752 | 2799 + 19 | in this study |
| | | TMV-rep-2223S | GCTGCGGTGTCTGAATCTCGTCAAG | 2223-2246 | 3304 | in this study |
| | | TMV-rep-1396S | TGGACAAATCTTTGTTACAATCCT | 1396-1419 | 4131 | in this study |
| | | TMV qFor1 | ATTAGACCCGCTAGTCACAGCAC | 5939-2961 | | in this study |
| | | TMV qRev1 | TAGAGTAGACGACGCAACGGTG | 6050-6071 | 131 | in this study |
| <i>Potato virus X (PVX)</i> | <i>Potexvirus</i> | PVX-F3 | AAGCTCCACAGGAAACACAG | 1612-1631 | | in this study |
| | | PVX-B3 | GGTTGGGAGTGTGAGTTCTT | 1790-1809 | 198 | in this study |
| <i>Potato virus Y (PVY)</i> | <i>Potyvirus</i> | GB2-YCPF1 | GCGCCGTCTCGCTCGA ATGGGAAATGACACAATCGATG* | 8571-8589 | | in this study |
| | | GB2-YCPR1 | GCGCCGTCTCGCTCAA AGCTCACATGTTCTTGACTCCAAG* | 9354-9374 | 804 + 19 + 19 | in this study |
| | | Mor1 | AGGAGGAAGCACTAAGAAG | 8591-8609 | 332 | [17] |
| | | Mor2 | CAAACCATAAGCCCATTTCATC | 8903-8923 | | [17] |
| | | MF05-21-R | CAATACGGTAAGTGCACACCCCG | 5654-5632 | 320 | [18] |
| | | MF05-22-F | CAGGGGCGTGAGTGGGGGGCTC | 5334-5354 | | [18] |
| | | TRSV-Pr-F | GGGGTGCTTACTGGCAAGG | 3195-3213 | 521 | [19] |
| | | TRSV-R | ACTTGTGCCCAGGAGAGCTA | 3716-3695 | | [20] |
| | | Nibfrg1F | GCACGGCAGTCTACGCACAAAC | 7174-7195 | 679 | in this study |
| | | Nibfrg1R | CTGGTAACGACTCCAACAAGCG | 7911-7890 | | in this study |
| <i>Turpin mosaic virus (TuMV)</i> | <i>Potyvirus</i> | TuMV-full-CPF | GTGTTTATCACCAGGCAGGTG | 8731-8751 | | [21] |
| | | TuMV-full-CPR | GTCTACCAGCATACAACCTTAC | 9627-9606 | 896 | [21] |

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|---|---------------------|----------------|------------------------------|----------------|------|---------------|
| <i>Turnip yellows virus (TuYV)</i> | <i>Polerovirus</i> | TuMV-F1qPCR | GTTCAACGCGGAAGCAGTTT | 9071-9090 | 208 | in this study |
| | | TuMV-R2qPCR | GCCTAAATGTGGGTTTGCG | 9279-9260 | | in this study |
| | | luteoviruses-F | GCTCTAGAATTGTTAATGACGGTCCG | generic primer | 610 | [22] |
| | | luteoviruses-R | CACGCGTCIACCTATTTIGGITTITG | generic primer | | [22] |
| | | TuYV-full-CPR | CCGGGTTCCCTCGTCTACCTA | 4095-4076 | 947 | in this study |
| | | TuYV-full-CPF | CACGTACGCGAAATCGTTAATG | 3148-3130 | 966 | in this study |
| <i>Barley yellow dwarf virus (BYDV)</i> | <i>Luteovirus</i> | TuYVR-K2 | ATTGGTCCTCGGCAACGTCG | 4114-4126 | | [23] |
| | | BYcpF | CCACTAGAGAGGTGGTGAAT | 2840-2859 | 640 | [24] |
| | | BYcpR | CCGATGTTGAGGAGTCTACC | 3479-3460 | | [24] |
| | | BYDV-PVinterF | GTTGAGTTTAAGTCACACGC | 3182 - 3201 | 294 | [25] |
| | | BYDV-Yan-Ra | TGTTGAGGAGTCTACCTATTTG | 3475 - 3454 | | [25] |
| | | BY5661R | TGCCGAACGTCTCTTCGAGTG | 5661-5640 | 825 | in this study |
| <i>Wheat streak mosaic virus (WSMV)</i> | <i>Tritimovirus</i> | BY4836F | ATCCTGGGAAACAGGCAGAAC | 4836-4856 | | in this study |
| | | WSMVcoatPRv | GAAACTGTGCGTGTCTCCC | 9197-9178 | 1014 | [26] |
| | | WSMVcoatPFv | GCGGTACGAATCGAGTGAG | 8161-8181 | | [26] |
| | | WSM8166 | GAGAGCAATACTGCGTGTACG | 8166-8186 | 743 | [27] |
| | | WSM8909 | GCATAATGGCTCGAAGTGATG | 8909-8929 | | [27] |
| | | WSMVspeFv | GCCTCGACA CGGGAGCTA | 8397-8417 | 354 | [28] |
| <i>Apple stem pitting virus (ASPV)</i> | <i>Foveavirus</i> | WSMVspeRv | ACC CATCCAGGAAGCAAGG | 8754-8736 | | [28] |
| | | ASPV-A | ATAGCCGCCCCGGTTAGGTT | 9237-9256 | 264 | [29] |
| | | ASPV-C | CTCTGAACCAGCTGATGGC | 8993-9012 | | [29] |
| | | ASPV forward | CWAAAYCCWTTTGAAACTGG | 8312-8220 | 839 | [30] |
| | | ASPV reverse | GCTTGCGTCCAAYTTTC | 9151-9134 | | [30] |
| | | ASPV sense | ATGTCTGGAACCTCATGCTGCAA | 8869-8895 | 370 | [31] |
| <i>Apple stem grooving virus (ASGV)</i> | <i>Capillovirus</i> | ASPV antisense | TTGGGATCAACTTTACTAAAAAGCATAA | 9211-9238 | | [31] |
| | | AGUV-2 | GGAATTCACACGACTCCTAACCCTCC | 6345-6371 | 499 | [32] |
| | | AGUV-U | CCCGCTGTTGGATTTGATACACCTC | 5873-5897 | | [32] |
| | | ASGV forward | GTTTGGAAGACGTGCTTC | 5644-5661 | 819 | [30] |
| | | ASGV reverse | ACACTAACCCGGAATGC | 6446-6463 | | [30] |
| | | ASGV sense | GTTTGGAAGACGTGCTTC | 6039-6064 | 272 | [31] |
| <i>Plum pox virus (PPV)</i> | <i>Potyvirus</i> | ASGV antisense | ACAATAACCCGGAATGC | 6286-6311 | | [31] |
| | | PPV-F3 | GGAATGTGGGTGATGATGG | 9 153-9 171 | 345 | [33] |
| | | PPV-RR | CTCTTCTGTGTTCCGACGTTTC | 9 475-9 497 | | [33] |
| | | mM3 | CATTTCCATAAACTCCAAAAGAC | 8 786-8 808 | 605 | [34] |
| | | mD5 | TATGTCACATAAAGCGTTCTC | 8 207-8 228 | | [34] |

| | | | | | | |
|---|--------------------|-----------------|---------------------------------|-------------|---------|------|
| <i>Apple chlorotic leaf spot virus</i> (ACLSV) | <i>Trichovirus</i> | PPV-RR | CTCTTCTTGTTCCGACGTTTC | 9 475–9 497 | 965 | [33] |
| | | RecJF | AATGATATTGATGATAGCCTTGAC | 8532-8556 | | [35] |
| | | 8RACLS | GCCTACAAATTAGGTGAGAGGCTC | 5 851–5 832 | 290 | [36] |
| | | 5FACLS | TTCCAATGGATCATGAGGTC | 5564-5 585 | | [36] |
| | | ACLSVFrII | CAAGAGAATTTTCAGTTTGCTCG | 6745-6766 | 791 | [30] |
| | | ACLSV antisense | AAGTCTACAGGCTATTTATTATAAAGTCTAA | 7507-7536 | | [31] |
| | | ACLSV sense | TTCATGGAAAGACAGGGGCAA | 6860-6880 | 647 | [31] |
| | | ACLSV antisense | AAGTCTACAGGCTATTTATTATAAAGTCTAA | 7507-7536 | | [31] |
| <i>Prune dwarf virus</i> (PDV) | <i>Ilarvirus</i> | PDVdpuF | CCGAGTGGATGCTTCACG | 1353–1370 | 220 | [35] |
| | | PDVdpR | CCTTTAATGAGTCCGT | 1572–1557 | | [35] |
| | | PDVcpF | CTTCCAACCTTCGACTGTTTG | 1143-1162 | 687 | [37] |
| | | PDVcpR | TCATCCACTGACTATTTTATCC | 1830-1809 | | [37] |
| | | PDVrna2F | TGATGACGTCGAAGAGCAAG | 550-569 | 418 | [37] |
| | | PDVrna2R | GGGACAAGTCGAAAGAGCAG | 949–968 | | [37] |
| General primers | pUPD2 plasmid | pUDP2-R2 | GAGGAAGCCTGCATAACG | n.a. | Various | [38] |
| | | pUPD2-F2 | CCCgatCAACTCGAGTGCCA | n.a. | | [38] |

* nontemplate bases in italics