Molecular assays to detect the presence and viability of *Phytophthora ramorum* and *Grosmannia clavigera*

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### RNA and DNA extractions

Fifty to 80 mg of wood scrapings were collected at each inoculation point from eight logs of four conifer species and used for the simultaneous extractions of gDNA and mRNA. Wood samples inoculated with *G. clavigera* were placed in 15 ml vials with two 10 mm stainless steel balls and were submerged in liquid nitrogen to keep the samples frozen. Vials were then placed in the Geno/Grinder (SPEX SamplePrep 2010, Metuchen, New Jersey, USA) at 15,000 rpm for 30 seconds. Wood samples inoculated with *P. ramorum* were hand-ground using a mortar and pestle. Mycelial samples were placed in Lysing Matrix C. All samples were flash-frozen in liquid nitrogen, ground in a FastPrep-24 homogenizer (MPBiomedicals) at 5.5 rpm for 30 seconds and re-submerged in liquid nitrogen. For *G. clavigera*, samples were removed from the freezer and submerged in liquid nitrogen to inhibit RNA degradation. Samples were individually placed in a mortar, immersed in liquid nitrogen and ground up into fine powder.

Simultaneous extraction of gDNA and RNA was performed using the AllPrep DNA/RNA Micro kit (QIAGEN Inc., Valencia, CA) following manufacturer instructions. Three extractions were performed for each as replicates. Genomic DNA concentration was measured using the Qubit fluorometer and all culture samples were diluted down to 1ng/µl using nuclease-free water. The concentration of RNA samples was measured using the NanoDrop 1000 spectrophotometer before diluting down to 10ng/ul. RNA integrity was assessed by band fluorescence using an agarose gel stained with ethidium bromide (EtBr).

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### cDNA synthesis, qPCR and RT-qPCR

Using the diluted RNA, cDNA synthesis was performed using the QuantiTect Reverse Transcription Kit (QIAGEN). Two µl of gDNA Wipeout buffer (7x), 10 ng of template RNA and a variable volume of RNase-free water for a total of 14 µl was used in the first step. Then 1 µl of Quantiscript Reverse Transcriptase, 4 µl of Quantiscript RT Buffer (5x) and 1 µl of RT primer mix was added to the gDNA eliminated solution.

Two TaqMan reactions per time point were performed for the measure of mRNA stability post heat treatment. This allowed differentiation of either gDNA or cDNA with their corresponding probes. Real-time PCR mix included 0.5X Quantifast Multiplex PCR MasterMix (QIAGEN), 400 mM of each forward and reverse primer, 20 mM of TaqMan probe and 2.2 ng of template (gDNA or cDNA) for a final volume of 10 µl. Thermal cycling parameters used were 5 minutes at 95oC for enzyme activation, followed by 40 cycles of denaturation at 95oC for 30 seconds and 60 seconds of annealing/extensions at 60oC. The threshold was automatically set and generated with the Applied Biosystems StepOneTM software; qPCR efficiency was also calculated automatically through the standard curves tab.

Table 1: Genes and assay sequences used for detection of *Phytophthora ramorum* and *Grosmannia clavigera*. Probe modification includes 5’ 6-FAM™ and 3’ Iowa Black® FQ quencher and fluorophore.

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| Species targeted | Gene | Function | Assay name[[1]](#footnote-1)[[2]](#footnote-2) | Sequence 5’ – 3’ |
| *P. ramorum* | PH178 | Predicted membrane protein | PH178\_F | TTTAGTCGGCTCTTATCCGGCATG |
| PH178\_R | CAGCAAGTAATAGAACAGGTTCCCCT |
| T3\_PH178\_EX | GCTTCAAGGAGAAAATTGCTCAGAACCA |
| PH178\_P | TCAGCTGGAGGATGGAGTTGACCCATGTT |
| PH218 | Chorismate mutase | T2\_PH218\_3F | ACCGGATCAACATCAACGATCAAATCA |
| PH218\_3R | ACTTGCCGAAATGGATACGCTTACT |
| T3\_PH218\_3EX | ATGACACCGCGTATGGCTCTACGG |
| T2\_PH218\_3P | CTGCGCAGTTGTTGCTCACTTGGGGGA |
| *G. clavigera* | MS359 | NAD-dependent 510-methylenetetrahydrafolate dehydrogenase | MS359\_F | AACAATGACCCTGCCGCA |
| MS359\_R | CGTCAATCGAGTCGTCGTTATTG |
| MS359\_EX | CTGCCAAGAGAATGGCTTCGCCTTC |
| MS359\_P | CTATGGTCAATTGGCTCTATGACTCGTGA |

1. Assay names ending with “\_F” and “\_R” refers to the direction of the oligonucleotides [↑](#footnote-ref-1)
2. Assay names ending with “\_EX” and “\_P” refers to the probe sequences [↑](#footnote-ref-2)