

MULTIPLEX PCR WORKSHEET

Quality and Quantity Test

DATE: _____

Technician: _____

PANEL

Supplies:

10X PCR Buffer Lot # _____

10mM dNTPs Lot # _____

50mM MgCl₂ Lot # _____

5 μM Platf/Platr primer mix Lot # _____

5 μM NS1/NS2 primer mix Lot # _____

Platinum Taq polymerase (5μ/ml) Lot # _____

dH₂O, Sterile Distilled Water

Ultra pure Agarose powder, Ethidium Bromide

Getting Started:

- _____ All reagents should be kept at -20°C. Take reagents out to thaw, vortex briefly, and spin down to collect the contents on the bottom. The Taq Polymerase is stored in glycerol and does not require thawing. All reagents should be kept on ice.
- _____ The master mix should be prepared in a laminar flow hood, biological safety cabinet, or in a room where PCR products are not generated or analyzed.
- _____ Use dedicated pipetters to prepare the master mix.
- _____ Change tips and gloves often.

<u>Location</u>	<u>Sample Number</u>	<u>Result</u>	<u>√</u>
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

Preparing the Sample

- _____ Determine the number of samples to be tested. Label a set of 1.5ml microcentrifuge tubes that will be used for 1:100 sample dilutions.
- _____ Determine the number of samples to be tested. Label a set of 0.2ml PCR reaction tubes for the Multiplex PCR reactions, include 3 additional tubes each for the controls: one each for the P. ramorum + control, healthy plant DNA control and non-template control (NTC), water used for DNA dilutions..