FTA Card Extraction Protocol for *Phytophthora infestans* DNA with High pH Steps

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Before beginning, turn on the water bath and set to 65˚C

1) Sterilize a hole puncher by spraying with 70% ethanol, flaming with a small bench top flame, and punching onto blank chromatography/filter paper. Make sure to sterilize the hole puncher in this way after every sample to prevent contamination.

2) Punch out a single 6mm disc from the applied dry sample spot (try to contain brown spots that may have been infected) while holding a 1.5mL microfuge tube underneath to catch the sample.

3) Add 400µL of FTA® purification reagent (Whatman Inc., USA) to each tube, vortex the samples, and incubate for 4 minutes at room temperature.

4) Discard the used FTA® purification reagent using a pipette, making sure that the disc stays in the tube.

5) Repeat the FTA® purification wash once more by following steps 3 & 4.

6) Add 400µL of a modified Te buffer (10mM Tris, 0.1mM EDTA buffer) to each tube, vortex the samples, and incubate for 4 minutes at room temperature.

7) Discard the used Te buffer using a pipette.

8) Repeat the Te buffer wash once more by following steps 6 & 7, making sure all buffer is removed and the disc remains. Transfer each disc to a new tube.

9) Add 70µL of alkaline incubation buffer (0.1 N NaOH, 0.3mM EDTA, pH 13.0) to each tube.

10) Centrifuge the samples slightly to make sure the disc is submerged in the solution.

11) Incubate the samples in a water bath for 5 minutes at 65˚C.

12) Add 130µL of neutralizing solution (0.1M Tris-HCL, pH 7.0) to each tube and vortex them in increments of a few seconds 5 times to mix. This will ensure that the reaction is stopped.

13) Incubate the tubes for 10 minutes at room temperature.

14) The FTA punches are left in the final solution and stored in the freezer (-20˚C) until use.

15) Solution eluted from the FTA cards is used for subsequent analysis.