

A High Throughput DNA Fingerprinting Tool for Biosurveillance of the Sudden Oak Death Pathogen *Phytophthora ramorum*

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Phytophthora ramorum has emerged repeatedly as four distinct clonal lineages in North America (lineages NA1, NA2, and EU1) and Europe (EU1 and EU2). Long-distance migration of *P. ramorum* is known to have occurred via the nursery trade. While most populations sampled in North American forests belong to the NA1 clonal lineage, nurseries have been shown to be infested with three lineages NA1, NA2 and EU1. EU1 and NA2 populations were discovered in environments around nurseries suggesting that nursery infestations could spread in the forest. It is therefore important to monitor populations for clonal lineages and screen for emergence of potential new lineages. To achieve this goal, we are developing a high throughput genomic tool that uses targeted sequencing to accurately identify species and lineage from minute amounts of pathogen material within a 24-hour time frame.

Targeted sequencing of a defined subset of the genome (<5Mb) allows PCR based enrichment of select genomic regions. This technique is powerful for environmental and outbreak samples given its robustness and speed. We selected our target genome regions in a hierarchical fashion using published gene sequences and unique markers generated by genome comparison (Feau and others 2018). Sequencing data from outbreak and survey samples can be used to accurately identify the pathogen, its lineage, and potential sources of introduction using primer panels that target unique regions.

We have developed two detection panels comprising 114 amplicons that can generate genome sequences polymorphic among *Phytophthora* species (panel I) or between *P. ramorum* lineages (panel II). We tested these panels on 28 samples and generated over 500 single nucleotide polymorphisms. We used variant calling, principal component analysis and phylogenetic assignment to accurately assign each sample to its phylogenetic clade and *P. ramorum* samples to the right lineage. This approach is scalable since each panel can be augmented as needed, and high-throughput as 384 samples can be pooled in a single reaction. The assay is suitable for *P. ramorum* outbreaks to facilitate an understanding of its spread and to enable early detection and control.

References

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