

Single-Strand Conformation Polymorphism Analysis of Ribosomal DNA for Detection of *Phytophthora ramorum* Directly From Plant Tissues¹

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Abstract

At the first Sudden Oak Death Science Symposium, we reported on the use of a single strand conformation polymorphism (SSCP) analysis for rapid identification of *Phytophthora ramorum* in culture. We have since assessed and improved the fingerprinting technique for detecting this pathogen directly from plant tissues. The improved SSCP protocol uses a single run PCR reaction with the same primer pair (ITS6/7) and consistently detects *P. ramorum* at 10 fg per reaction or above. It provides reliable diagnoses of *P. ramorum*, whether it is a single infection or dual infection (a second *Phytophthora* species involved). This technique also can provide accurate diagnoses of diseases caused by 12 other species of *Phytophthora* without additional work. These species (*P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. lateralis*, *P. megasperma*, *P. nemorosa*, *P. nicotianae* and *P. pseudosyringae*) are common in ornamental plant and forest tree nurseries, as well as in natural forested environments. The through-put capacity of this technique can be greatly improved by use of fluorescence-based technologies, such as those common to most commercially available DNA sequencers. This study provides an alternative protocol with increased detection scope and accuracy, at a reduced cost, for future surveys of nurseries, parks and forests for *Phytophthora* species.

Key words: *Phytophthora ramorum*, single strand conformation polymorphism, sudden oak death

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