

Assessment of a Portable, Inexpensive, Rapid Amplification Platform for Loop-mediated Isothermal Amplification (LAMP)

Phytophthora ramorum is the causal agent of sudden oak death in forests on the west coast of the United States, and dieback and leaf blight in a wide range of woodland trees, shrubs, and herbaceous plant species in the UK and other areas of Europe. Suitable detection methods are needed in order to facilitate effective measures for the control and eradication of this pathogen. In particular, rapid methods that can be carried out in the field, minimizing the delay between sampling and diagnosis, could help to reduce the spread of *P. ramorum*. Lateral flow devices can be used to identify members of the genus *Phytophthora* in the field, but identification at the species level requires a nucleic acid-based test.

A loop-mediated isothermal amplification (LAMP) assay using six primers (annealing to 8 regions) was developed for the detection of *P. ramorum* in plant material. The sensitivity and specificity of this method are similar to those of real-time PCR methods for detection of this pathogen, with the advantages that LAMP does not require thermal cycling and the reaction is completed in 1 hour. LAMP assays have subsequently been developed at Fera for other plant pathogens, including *P. kernoviae* and *Botrytis cinerea*.

LAMP products can be detected in the laboratory by gel electrophoresis, or in the field by observation of precipitated magnesium pyrophosphate or a colour change on the addition of an intercalating dye. However, these methods require the reaction tubes to be opened after amplification, which can result in carry-over contamination. Given that a LAMP reaction generates of the order of 25 µg of amplicon, five to ten more than a standard PCR, it is very important to avoid opening the finished product reaction tube.

The GENIE detection system developed and manufactured by OptiGene allows real-time LAMP to be performed on a low-power portable platform, suitable for use in the field. The closed-tube nature of GENIE avoids any post-amplification handling thereby eliminating laboratory contamination with the amplified product.

The GENIE system consists of two independent heating blocks, each block taking up to eight 0.2ml microtubes and offering a gradient facility for optimisation of the LAMP reaction temperature.

GENIE measures the fluorescence, in real time, of an intercalating dye during the amplification process. This allows the reaction to be monitored and the time of initial amplification gives an indication of template copy number.

From melt curves LAMP reactions primarily consist of a single amplified product usually melting between 83 – 89°C. This is several degrees higher than one sees for standard PCR products. It has been found that by performing an annealing curve on the finished amplification product one sees a single product peak from which one can extract a T_a value. Similar to a melt curve, this annealing T_a value is unique to the sequence amplified, thus confirming amplification of the correct product.

The use of fast isothermal amplification reagents using a novel *Geobacillus sp.* Large Fragment DNA polymerase allows results to be obtained in less than 30 minutes. DNA extracted from a culture of *P. ramorum* tested positive by real-time LAMP in as little as 12 minutes in contrast with LAMP using NEB *Bst* Large Fragment DNA polymerase which typically takes around 1 hour.

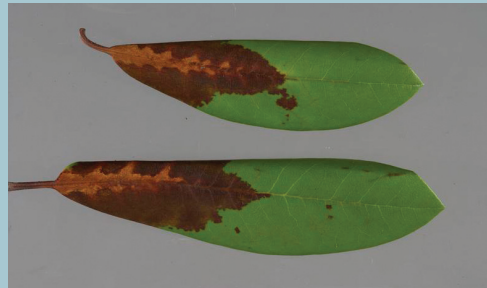


Figure 1. *Phytophthora ramorum* on *Viburnum* sp. leaf

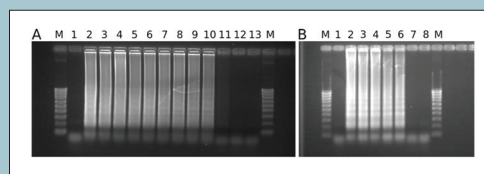


Figure 2. Sensitivity and specificity of *Phytophthora ramorum* LAMP assay. A. A dilution series of *P. ramorum* DNA amplified by LAMP. M: marker; lane 1: negative control (water); lanes 2 and 3: 10 ng; lanes 4 and 5: 1 ng; lanes 6 and 7: 100 pg; lanes 8 and 9: 50 pg; lanes 10 and 11: 10 pg; lanes 12 and 13: 1 pg. B. Cross reactivity of LAMP assay with *P. lateralis* DNA. M: marker; lane 1: negative control (water); lane 2: positive control (10 ng *P. ramorum* DNA); lanes 3 and 4: 70 ng *P. lateralis* DNA; lanes 5 and 6: 7 ng *P. lateralis* DNA; lanes 7 and 8: 700 pg *P. lateralis* DNA



Figure 3. The OptiGene GENIE II detection system

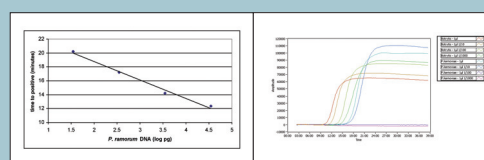


Figure 4. Time to positive result for a dilution series of *P. ramorum* DNA on the GENIE detection system

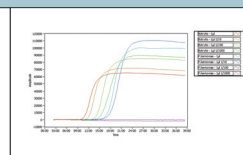


Figure 5. Amplification plots for real-time LAMP performed on the GENIE detection system

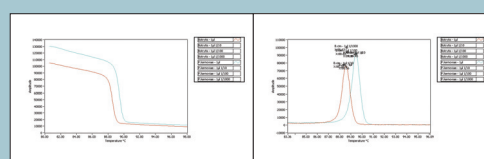


Figure 6. Melt curve analysis on the GENIE detection system



Authors

Jenny Tomlinson¹

Neil Boonham¹

Duncan Clark²

Stephen Millington²

Michael Andreou²

Anna Siddle²

Addresses

¹ The Food and Environment Research Agency
Sand Hutton, York.
YO41 1LZ. UK.

² OptiGene Ltd,
Unit 5,
Blatchford Road, Horsham,
West Sussex, RH13 5QR. UK.

References

Tomlinson J.A., Barker I., and Boonham N. 2007. Faster, simpler, more-specific methods for improved molecular detection of *Phytophthora ramorum* in the field. *Applied and Environmental Microbiology* 73:4040-4047.

Acknowledgement

The authors wish to acknowledge funding from Plant Health Division of Defra and from the Proof of Concept fund of the InterAct Partnership



Fera is an Executive Agency of Defra

www.defra.gov.uk/fera