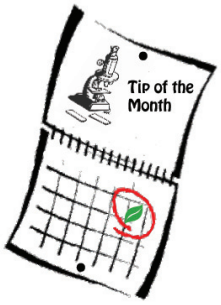

Loop-mediated Isothermal Amplification (LAMP) as a Diagnostic Tool

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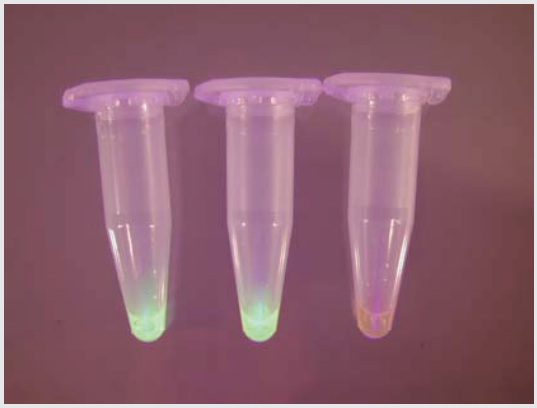
As a diagnostician I am always looking for assays that are rapid, accurate, easy to use, and inexpensive. Once I found out it is possible to have the specificity of PCR without the need for thermal cyclers, gels, or expensive and short-lived fluorescent probes using the method known as LAMP, I was definitely interested. Loop-mediated isothermal amplification (LAMP) takes place in a single microfuge tube and requires only a water bath or heat block. Results are evaluated visually without extensive manipulation of product. Since the method is the equivalent of a muscle car as far as speed (from a few copies of DNA to 10^9, optimally, in less than an hour) and is highly specific, it seems to have the qualities that would be extremely useful in a diagnostic laboratory. We have developed an assay for detection of *Rhodococcus fascians* using this method (Serdani, *et al*., 2009), are currently using LAMP for fire blight detection (Temple *et al*., 2008), and have been very happy with both tests. The assay can be used on host tissue in addition to pure cultures, and it lives up to the claims for speed and selectivity. The following information is provided to pique your interest in developing your own assays.

LAMP was developed by Tsugunori Notomi of Eiken Chemical Company and colleagues at the University of Tokyo and Osaka University Medical School. Their seminal paper was published over ten years ago (Notomi *et al*, 2000), and the method has since been adapted for use with fungi (Tomlinson, *et al*, 2010), nematodes (Kikuchi, *et al*., 2009), and viruses (Fukuta *et al*. 2003) in addition to phytobacteria. The process takes place in a microfuge tube containing a small amount of extracted sample DNA and four primers that are specific to six regions of your target DNA. A strand-displacing polymerase is used at 60-65°C (the specific temperature used depends on system optimization) and a series of looped structures result. The rather complex process of amplification and the structure formation is detailed at the Eiken website, which also includes an animation of the reaction (http://loopamp.eiken.co.jp/e/lamp/index.html). As a by-product of amplification, magnesium pyrophosphate accumulates in proportion to the amount of the specific product formed, and can be seen as turbidity in the reaction tube. If it is difficult to see this turbidity, a DNA binding dye may be used, which will integrate with the amplified product. This dye-DNA complex will fluoresce under UV light, which makes it easier to distinguish between positive and negative reactions.



Positive LAMP reactions are shown in the two tubes on the left. The tube on the right is a negative reaction. The image was taken under UV illumination and fluorescence is due to addition of PicoGreen to all tubes.

For our LAMP assay for *Rhodococcus fascians*, we use either the FastSpin or Quiagen kits to extract DNA when starting with plant material, or if using a pure culture, simply boil the bacteria briefly to extract DNA. An aliquot is then added to the reaction mixture, the tubes are incubated at 60°C, and visualization is with PicoGreen (or the fluorescent dye of your choice). The entire process is

completed in less than 3 hours. We have found the process to be fast, accurate, and very simple to use. However, the reaction products are extremely stable and can contaminate the laboratory in a persistent manner if you are not careful when opening the tubes to add the dye. This is best done in a room separate from where the DNA extraction occurs and where the reaction mixture is prepared.

There are web sites that can help you to design your own primers, one of which is at http://primerexplorer.jp/elamp4.0.0/index.html, and excellent technical information at the Eiken website: http://loopamp.eiken.co.jp/e/lamp/index.html.

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National Plant Protection Laboratory Accreditation Program and 2011 *P. ramorum* Proficiency Test Panel

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The National Plant Protection Laboratory Accreditation Program (NPPLAP) evaluates laboratories using molecular diagnostics on behalf of USDA-PPQ regulatory programs to ensure their capability for making accurate diagnostic determinations. In addition to ensuring lab capability within PPQ and other agencies in the USDA, NPPLAP engages the National Plant Diagnostic Network (NPDN) in this process to increase diagnostic capacity and proficiency. The goal of this program is to establish a state of readiness when needed by PPQ in emergency situations. NPPLAP also fosters the adoption of practices that promote continuous improvement and accreditation standards suitable for use by plant diagnostic labs and serves to develop functional Quality Assurance programs. NPPLAP began as the Provisional Approval process for the USDA *Phytophthora ramorum* Emergency Program and currently accredits laboratories to diagnose *Phytophthora ramorum* and the Huanglongbing (Citrus Greening) pathogen, with over 30 labs participating in one or both programs. The NPPLAP website is: www.aphis.usda.gov/plant_health/cphst/npplap.shtml. This website describes the program in detail and contains links to Inspection Checklists and other NPPLAP-related documents.

Visit the NPDN diagnostic website at www.npdn.org/diagnostics to read the 2011 *Phytophthora ramorum* panel information letter and to download a form for requesting panels. The documents can be found under the heading "Provisionally Approved Laboratories."