MULTIPLEX PCR-DNA KIT FOR EARLY DETECTION AND IDENTIFICATION OF *Ganoderma* SPECIES IN OIL PALM

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MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2010 MPOB TS No. 73

Polymerase chain reaction (PCR) is being used to diagnose plant pathogens, including the detection and quantification of different plant pathogenic fungi, oomycetes, bacteria, nematodes, viruses and biocontrol agents (Lievens *et al*,. 2006). This method is very sensitive and is capable of monitoring and quantifying the presence of a pathogen in plant tissues before the symptoms appear. Identification of ribosomal ribonucleic acid (rRNA) genes is the most appropriate method to study and identify fungal species in different fungal taxa at many levels (Moncalvo *et al*., 1995; Utomo and Niepold, 2000). The availability of a rapid, inexpensive and accurate diagnostic technique, which is specific and readily adapted to large-scale testing for the early detection of *Ganoderma* in oil palm, would benefit decision-making for appropriate control (Idris and Ariffin, 2004). *Ganoderma* is the causal agent of the basal stem rot (BSR) disease in oil palm.

Technologies for the early detection of *Ganoderma* have been achieved through a culturing technique using *Ganoderma* selective medium (GSM) (Ariffin and Idris, 1991), through the molecular polymerase chain reaction-deoxyribonucleic acid (PCR-DNA) technique (Idris *et al*., 2004) and the enzyme-linked immunosorbent assay-polyclonal antibody (ELISA-Pab) protocol (Idris and Rafidah, 2008). These techniques offer several advantages in providing specificity and sensitivity in *Ganoderma* detection in oil palm. Utilization of the Multiplex PCR-DNA Kit for the early detection and identification of four species of *Ganoderma*s in oil palm is offered to the oil palm industry.

PROCEDURES

A summary of the process for the early detection and identification of *Ganoderma* species using the Multiplex PCR-DNA Kit is presented in *Figure 1*.

The Multiplex PCR-DNA Kit is a qualitative *in vitro* test with enhanced sensitivity and specificity which utilizes the dual priming oligonucleotide (DPO) technology (Chun *et al*., 2007). The technique allows for early detection of and differentiation between the four species of *Ganoderma* in oil palm (Idris, 1999), namely, *G. boninense*, *G. zonatum*, *G. miniatocinctum* and *G. tornatum* in a single reaction (*Figure 2*). The first three species are pathogenic to oil palm while the latter is non-pathogenic.

The DPO technology provides freedom in primer design and PCR optimization, which maximizes PCR specificity and sensitivity by dramatically eliminating non-specific priming. The Multiplex Kit consists of a 2X Multiplex Master Mix, a 5X Ganoderma4 PM and an 8-methoxypsoralen (8-MOP) system to extinguish the template activity of contaminating DNAs. The 8-MOP is also known to intercalate into double-stranded nucleic acid, and form a covalent interstrand crosslink with incident light of 320-400 nm wavelength after photo-activation. UV irradiation (365 nm) for 20 min will prevent the carry-over of contamination onto the amplified PCR products. The addition of an internal control is to identify the processed samples that may interfere with PCR amplification. The internal control is a DNA plasmid, which is introduced into each amplification reaction, and is co-amplified with target DNA from the field sample. Field studies have confirmed that the Multiplex PCR-DNA Kit is able to detect and identify the presence of *Ganoderma* species in oil palm as shown in *Figure 3*.

SERVICE PROVIDED

MPOB offers the service of early detection and identification of *Ganoderma* species in oil palm. This service is offered to researchers interested in verifying the four species of *Ganoderma* associated with BSR disease, which are *G. boninense*, *G. zonatum*, *G. miniatocinctum* and *G. tornatum*.

ISSN 1511-7871
9 771511 787001

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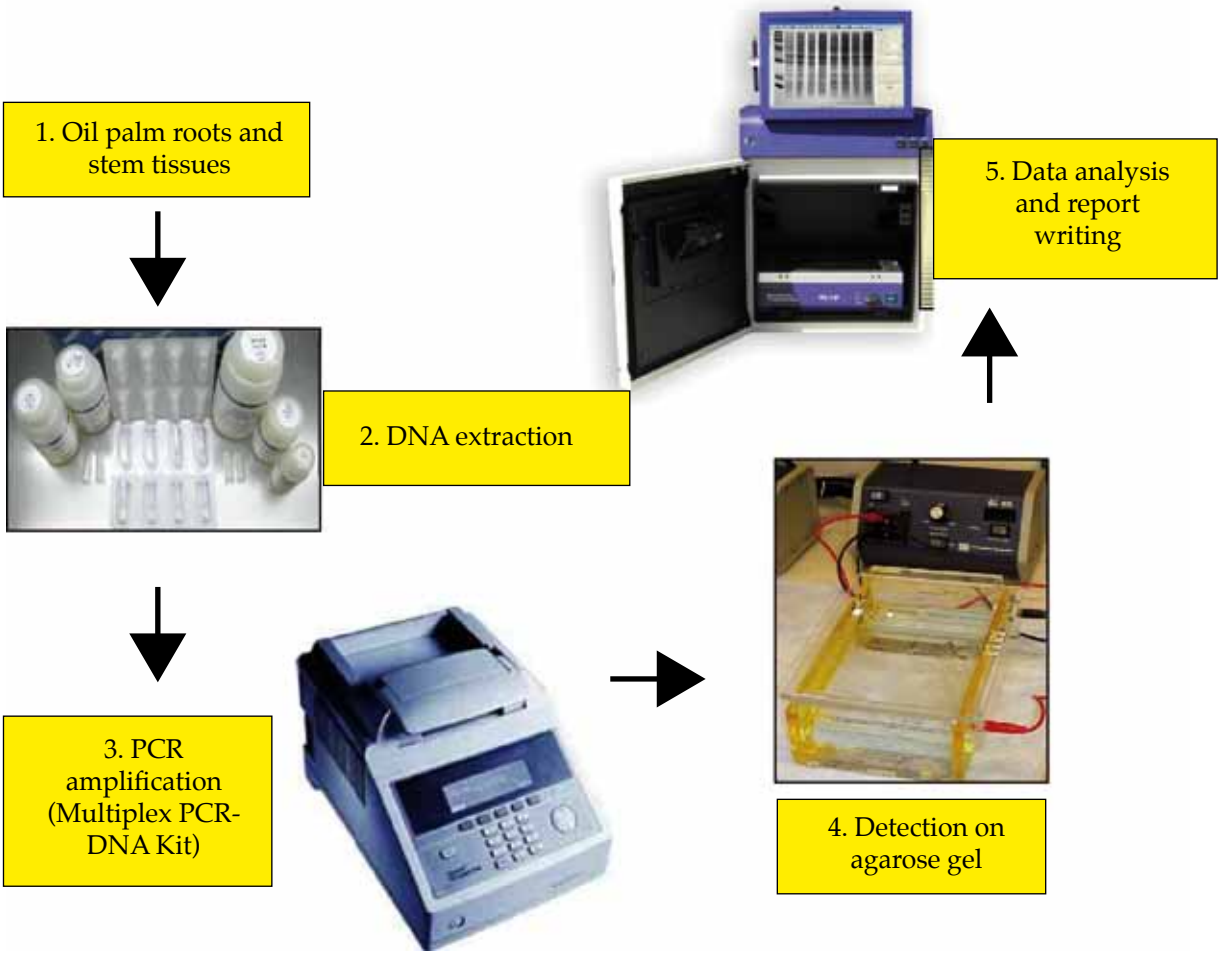

Figure 1. Process in the early detection of Ganoderma *using the Multiplex PCR-DNA Kit.*

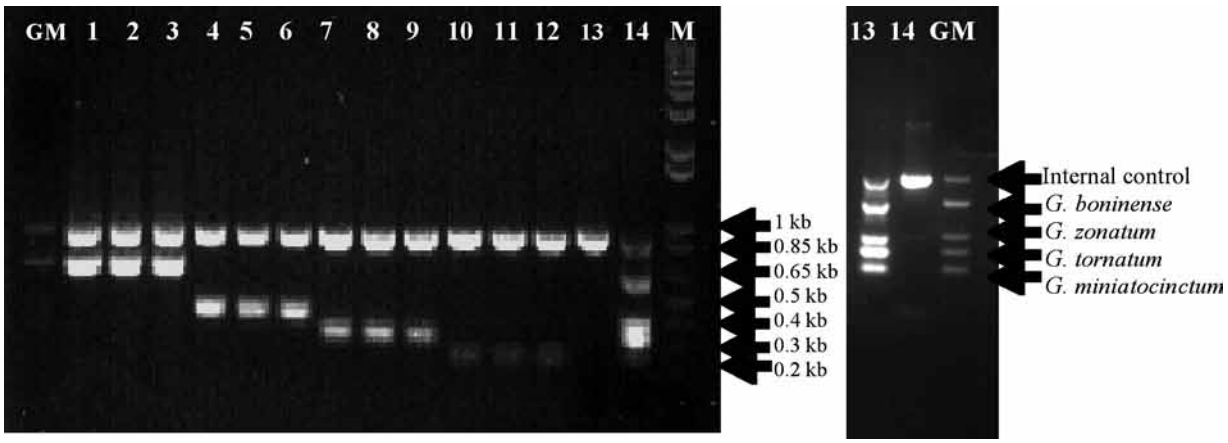

Figure 2. Amplification of polymerase chain reaction (PCR) products for early detection and identification of Ganoderma *species using the Multiplex PCR-DNA Kit. Note: Ganoderma 4 Marker (lane GM); DNA extracted from* G. boninense *(lanes 1, 2 and 3);* G. zonatum *(lanes 4, 5 and 6);* G. tornatum *(lanes 7, 8 and 9);* G. miniatocinctum *(lanes 10, 11 and 12); positive control (Lane 13); negative control (lane 14); and 1 kb ladder (lane 15).*

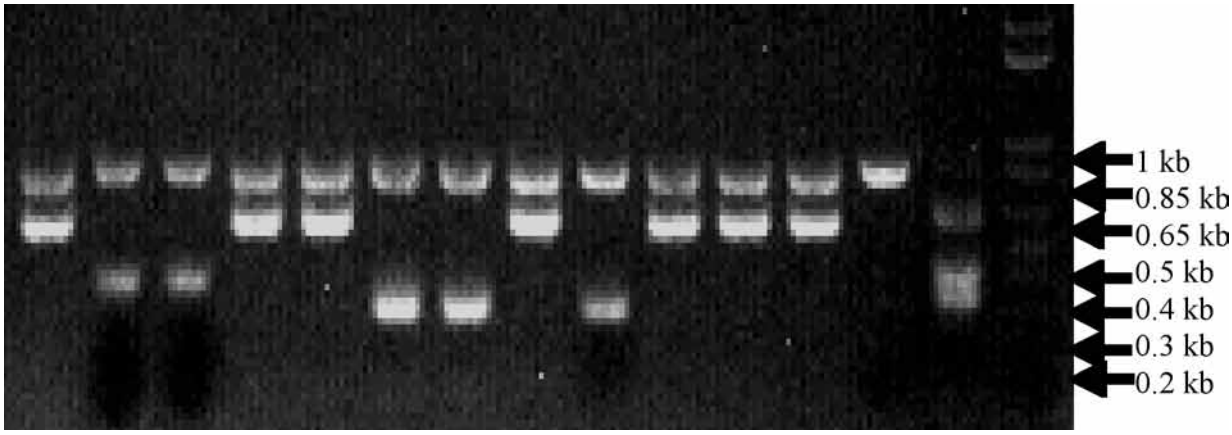

Figure 3. Amplification of Polymerase chain reaction (PCR) products from DNA of Ganoderma isolated from an oil palm field using the Multiplex PCR-DNA Kit. Note: G. boninense *(lanes 1, 4, 5, 8, 10, 11 and 12);* G. zonatum *(lanes 2 and 3);* G. tornatum *(lanes 6, 7 and 9); negative control (lane 13); positive control (lane 14); and 1 kb DNA ladder (lane M).*

BENEFITS AND COST

The service offered is a reliable method to detect and identify *Ganoderma* species in field samples. The service is inclusive of DNA extraction, PCR amplification, detection on gel and interpretation of the results.

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