# Diagnostic markers for the identification of the tree species *Shorea leprosula* Miq. and *S. parvifolia* Dyer and the geographic origin of *S. leprosula* Miq.

Dissertation

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# 1. Introduction

## 1.1 Background of the study

Deforestation is estimated at a rate -0.18 per year during 2000-2005 (FAO, 2009). Logging is responsible for about one third of the total global deforestation and it is estimated that more than half of all logging activities are conducted illegally (Brack 2003). Illegally sourced timber competes with sustainable produced timber and causes market distortions (Marijnissen 2003). Control of illegal activities in timber production and world wide trade has received increasing attention over recent years. Forest certification was developed as a market-based response to address consumer concern about deforestation and the quality of forest management (Wingate and McFarlane 2005).

Forest certification plays an important role to develop and implement sustainable forest management practices. There are four major certification schemes: the Canadian Standards Association National Sustainable Forest Management Program (CSA; http://www.csa-international.org), the Forest Stewardship Council (FSC; http://www.fsc.org/fsc), the Programme for the Endorsement of Forest Certification (PEFC; http://www.pefc.org) and the Sustainable Forestry Initiative (SFI; http://www.sfiprogram.org) (Wingate and McFarlane 2005). Different certification scheme of timber from sustainable managed forest should be supported by system to certify and verify the legality of timber. One main component of all certification schemes is the Chain of Custody (CoC) (Dykstra et al. 2003a).

Chain of custody (CoC) is the process of tracking and recording the possession and transfer of wood and fiber from a certified forest through the different stages of production –primary manufacturer, second manufacturer, wholesaler, and retailer– and to the end user (Dykstra et al. 2003b). CoC provides additional evidence that forest products originated from certified-forests. Timber identification of tree species and their origin is technically essential for monitoring, control and thus enforcement of species trade regulations (Wingate and McFarlane 2005).

Identification of timber species has been done using wood anatomical methods (Koch et al. 2008), however it is often difficult to identify species solely based on wood anatomy and

sometimes impossible in the case of taxonomically related species. Log tracking systems have been developed from painting systems through the use of bar-code labels, mechanised coding systems and radio-frequency identification transceivers (Dykstra et al. 2003a, Dykstra et al. 2003b), but these methods can be manipulated. Operators can remove labels and replace them with their own, also bar-coded labels can be difficult to scan and 5% of such labels will be dislodged by handling (Dykstra et al. 2003a).

There is a need for better control methodologies, and genetic fingerprints could play an important role in this respect, since the manipulation of the DNA is impossible (Finkeldey et al. 2008a). Genetic fingerprinting methods can be used after two basic requirements are fulfilled: (i) protocols for DNA isolation from wood of different age and processing status are deve-loped and (ii) diagnostic markers for the identification of timber species and timber origins are available (Finkeldey et al. 2008b).

Wood can become a suitable tissue for the control of the timber and wood trades. The DNA extraction methods from dry wood have been developed due to the interest in timber tracking (De Filippis and Magel 1998, Dumolin-Lapègue et al. 1999, Deguilloux et al. 2002, Rachmayanti et al. 2006, Yoshida et al. 2007). Molecular tools for the certification of dipterocarp wood have been promoted by: (i) the development of protocols for DNA isolation from dipterocarp wood (Rachmayanti et al. 2006, Rachmayanti et al. in press), (ii) study of chloroplast and nuclear marker DNA (Indrioko et al. 2006, Cao et al. 2006a, 2006b, 2009, Nguyen in prep.) and (iii) the development of specific PCR (Polymerase Chain Reaction) markers / SCAR (Sequence Characterized Amplified Region) markers (present study).

## 1.2 Investigated species

*Shorea parvifolia* Dyer and *S. leprosula* Miq. (Dipterocarpaceae) are the most dominant trees of mixed dipterocarp forests in Indonesia (Ashton 1982, Newman et al. 1996a, 1996b). They are abundant on deep clay soils at elevations below 700 m asl (Newman et al. 1996a, 1996b). Like other trees of the family Dipterocarpaceae, *S. leprosula* and *S. parvifolia* form large tree, reaching 60 m high and are a commercially popular timber in Indonesia. The timber of *S. leprosula* and *S. parvifolia* is classified as light hardwood of the red meranti group (Newman

et al 1996a, 1996b). Circa 50% of the world's demand for plywood has been supplied by Indonesia (FAO 2002), principally from red meranti (*Shorea* section *Rubroshorea*) (Abe 2007). Furthermore, WWF (World Wildlife Fund) estimated that 83% of total timber production in Indonesia come from illegal logs (Tacconi et al. 2004).

Amplified fragment length polymorphisms (AFLPs) markers have been used to study the genetic diversity within and among populations of *S. leprosula* and *S. parvifolia,* particularly in Sumatra and Borneo (Cao et al. 2006a). The study showed that *S. leprosula* is genetically more variable than *S. parvifolia* at the population level and species level ($H_{ep}$= 0.161 for *S. leprosula* and 0.138 for *S. parvifolia*, $H_{es}$ = 0.211 for *S. leprosula* and 0.205 for *S. parvifolia*). Genetic differentiation among populations was high for both species ($G_{ST}$ = 25% for *S. leprosula* and 31% for *S. parvifolia*). The genetic differentiation between islands was significant for *S. leprosula*, but not for *S. parvifolia*. Several highly differentiating AFLP markers were identified as potentially diagnostic markers to differentiate between the islands Borneo and Sumatra and between other regions. Other AFLP markers are potentially useful to identify the species *S. leprosula* and *S. parvifolia*.

AFLP combines the specifity of restriction enzymes with the specificity of the PCR (Vos et al. 1995). This technique requires no specific *a priory* sequence knowledge and is a reliable method to generate hundreds of informative genetics markers. Sequencing of AFLP fragments will provide information on the nucleotide sequence. Variation among nucleotide sequences can be used to assess differentiation patterns among species (Obayashi et al. 2002), genera (Kamiya et al. 2005) or even higher taxonomic levels (Dayanandan et al. 1999, Yulita et al. 2005). However, AFLP markers are less suitable for single locus assays because of its intensity of labour, high costs and the redundant information content (Brugmans et al. 2003). Furthermore, AFLP fingerprints rely on the availability of high quality DNA (McLenachan et al. 2000). Degraded DNA such as DNA isolated from wood is unlikely to be suitable for AFLP analyses. Therefore, there is a strong need to convert AFLP fragments into convenient and inexpensive single locus-PCR based markers, such as sequence characterized amplified region (SCAR) markers.

The conversion of AFLP markers from multilocus fingerprints to single locus PCR markers, known as SCAR (Sequence Characterized Amplified Region) markers, makes them potentially useful as diagnostic markers (figure 1). Bands of interest may be excised from the

gel, clonned and transformed into SCAR markers by designing specific primers (Paran and Michelmore 1993).

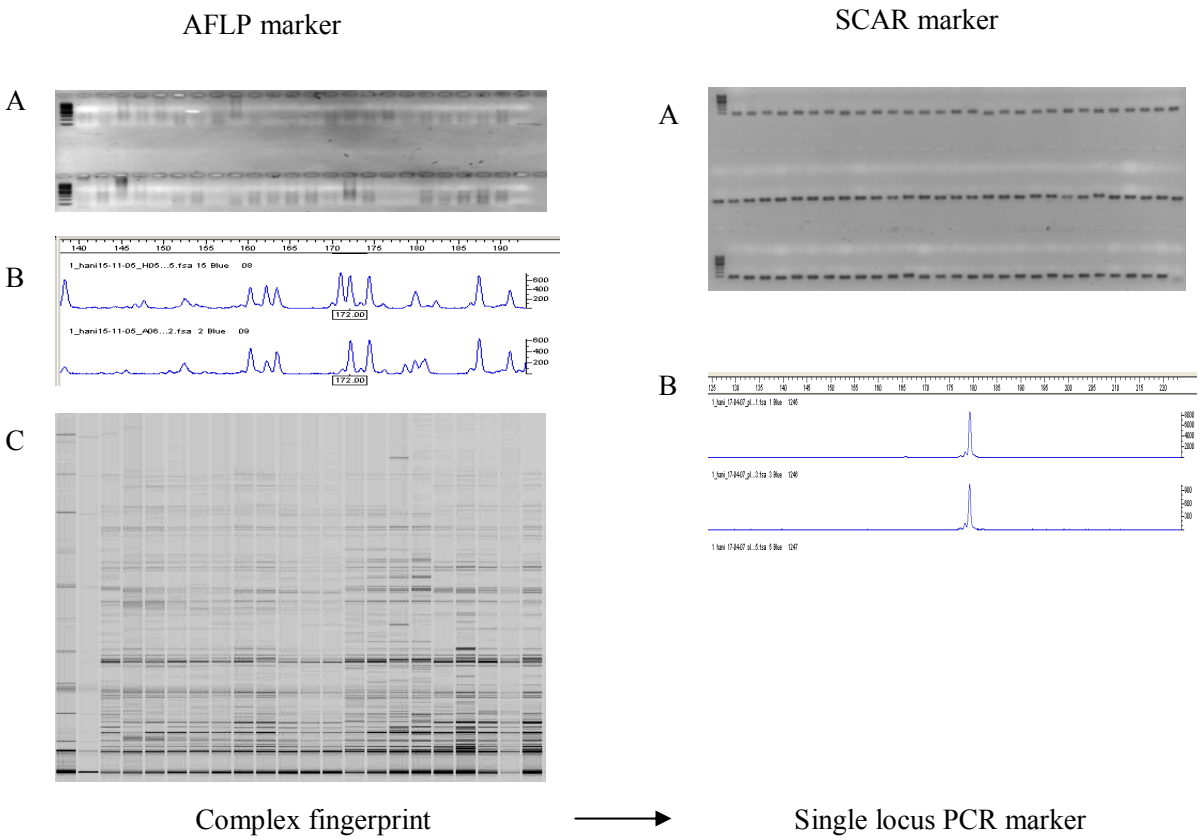


Figure 1: Visualization of AFLP and SCAR marker. (A) Agarose gel, (B) Capillary electrophoresis, (C) Polyacrilamide gel. Photo AFLP-A from Cui-Ping Cao.