



**UNIVERSITI PUTRA MALAYSIA**

**DNA FINGERPRINT DATABASES OF CHENGAL (*Neobalanocarpus  
heimii*)  
FOR FORENSIC FORESTRY INVESTIGATIONS**

**TNAH LEE HONG**

**IB 2007 2**



**DNA FINGERPRINT DATABASES OF CHENGAL  
(*Neobalanocarpus heimii*) FOR FORENSIC  
FORESTRY INVESTIGATIONS**

**TNAH LEE HONG**

**MASTER OF SCIENCE  
UNIVERSITI PUTRA MALAYSIA**

**2007**



**DNA FINGERPRINT DATABASES OF CHENGAL (*Neobalanocarpus heimii*)  
FOR FORENSIC FORESTRY INVESTIGATIONS**

**By**

**TNAH LEE HONG**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Master of Science**

**July 2007**



**Specially dedicated to my loving husband and family members...**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment  
of the requirements for the degree of Master of Science

**DNA FINGERPRINT DATABASES OF CHENGAL (*Neobalanocarpus heimii*)  
FOR FORENSIC FORESTRY INVESTIGATIONS**

By

**TNAH LEE HONG**

**July 2007**

**Chairman : Faridah Qamaruz Zaman, PhD**

**Institute : Bioscience**

Illegal logging poses a significant threat to the sustainability of Malaysian forest ecosystems. Presently, foresters have to depend on wood anatomical evidences to link the suspected timber thefts to the source trees but this is inconclusive. This study was aimed to utilize DNA markers in plant DNA fingerprinting for forensic applications using *Neobalanocarpus heimii* as a model. To generate a comprehensive DNA database of *N. heimii* for individual identification, 30 natural populations were identified from 27 forest reserves, and a total of 1081 individuals were collected throughout Peninsular Malaysia. An extensive evaluation of 51 short tandem repeat (STR) loci developed for Dipterocarpaceae managed to identify 12 STR loci, which showed specific amplification, absence of null alleles, single-locus mode of inheritance, and absence of mononucleotide repeat motifs in *N. heimii*. Cluster analyses via assignment test and genetic distance divided the 30 populations into three genetic clusters, corresponding to three geographical regions: Region A (west), Region B (central and south) and Region C (northeast). DNA databases of *N. heimii* were constructed and characterized at the levels of population, region and Peninsular Malaysia. Independence tests showed that the majority of the loci significantly

deviated from Hardy-Weinberg equilibrium due to population substructuring and inbreeding. Thus, the match probability of *N. heimii* should be estimated using the ‘subpopulation-cum-inbreeding model’ that adjusted for coancestry ( $\theta$ ) and inbreeding ( $f$ ) coefficients. The conservativeness tests showed that both the regional and Peninsular Malaysian databases were conservative and should be adequate to predict allele and genotype frequencies of *N. heimii* throughout Peninsular Malaysia. With a combined power of discrimination of more than 0.9999999999999999, the Peninsular Malaysian database should be able to provide legal evidences for court proceedings against illegal loggers on *N. heimii*. The comprehensive DNA fingerprinting databases developed for *N. heimii* are the first reported for a tropical tree species and the methodology developed should be able to serve as a model for the study of other important timber species in Malaysia. The availability of DNA fingerprinting databases for the majority of important timber species in Malaysia would enhance the capacity of Forest Department officials to curb the problem of illegal logging and this would indirectly ensure the conservation and sustainable utilization of forest resources in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PANGKALAN DATA PEMPROFILAN DNA CHENGAL (*Neobalanocarpus heimii*) UNTUK PENYIASATAN FORENSIK PERHUTANAN**

Oleh

**TNAH LEE HONG**

**Julai 2007**

**Pengerusi: Faridah Qamaruz Zaman, PhD**

**Institut: Biosains**

Pembalakan haram memberikan ancaman yang ketara ke atas kemampuan ekosistem hutan di Malaysia. Buat masa kini, pegawai hutan bergantung kepada bukti anatomi kayu untuk mengaitkan pembalok haram yang disyaki dengan punca pokok yang ditebang. Akan tetapi, bukti sedemikian adalah tidak memadai. Tujuan kajian ini adalah untuk mengaplikasikan penanda DNA dalam pemprofilan DNA tumbuhan untuk forensik perhutanan. Untuk tujuan ini, *Neobalanocarpus heimii* telah dijadikan sebagai model. Bagi penjana pangkalan data yang terperinci, 30 populasi semulajadi *N. heimii* dikenalpasti dari 27 hutan simpan di Semenanjung Malaysia dan sejumlah 1081 individual telah disampel. Penilaian terperinci ke atas 51 lokus jujukan ulangan pendek (STR) yang dijana untuk Dipterocarpaceae mengenalpasti 12 lokus yang sesuai diguna pakai untuk *N. heimii*. Kesemua lokus yang dikenalpasti ini menunjukkan ciri-ciri berikut: amplifikasi yang spesifik, ketiadaan alel nul, mod pewarisan mengikut Hukum Mendel, dan ketiadaan jujukan ulangan mononukleotida. Analisis kelompok melalui ujian penugasan dan jarak genetik membahagikan 30 populasi kepada tiga kelompok genetik, sejajar dengan tiga kawasan geografi, iaitu Kawasan A (barat), Kawasan B (tengah dan selatan) dan

Kawasan C (timur-utara). Pangkalan data pemprofilan DNA bagi *N. heimii* telah dijana berdasarkan populasi, kawasan dan Semenanjung Malaysia. Ujian kebebasan yang dilakukan ke atas semua pangkalan data ini menunjukkan kebanyakan lokus menyimpang secara signifikan daripada keseimbangan Hardy-Weinberg disebabkan oleh substruktur populasi dan biakbaka dalaman. Oleh yang demikian, kebarangkalian kesamaan genotip *N. heimii* perlu dianggar menggunakan model substruktur populasi dan biakbaka dalaman yang diperbetulkan dengan pemalar kesamaan keturunan ( $\theta$ ) and biakbaka dalaman ( $f$ ). Ujian kekonservatifan menunjukkan pangkalan data kawasan dan Semenanjung Malaysia adalah konservatif dan memadai untuk penganggaran frekuensi alel dan genotip *N. heimii* di seluruh Semenanjung Malaysia. Dengan kuasa diskriminasi melebihi 0.9999999999999999, pangkalan data pemprofilan DNA Semenanjung Malaysia dapat memberi bukti yang kukuh di mahkamah untuk penyiasatan kes pembalakan haram *N. heimii* di Semenanjung Malaysia. Penjanaan pangkalan data DNA ke atas *N. heimii* yang komprehensif ini merupakan kajian yang pertama dilaporkan dalam spesis pokok tropikal. Maka, kaedah ini diharap dapat dijadikan model untuk penjanaan pangkalan data DNA bagi spesis kayu balak lain yang penting di Malaysia. Dengan kewujudan pangkalan data DNA ini, kapasiti Jabatan Hutan dalam menanagani masalah pembalakan secara haram akan dipertingkatkan dan ini secara tidak langsung akan memastikan keberkesanan dalam pemuliharaan dan penggunaan sumber hutan di Malaysia.



## ACKNOWLEDGEMENTS

First and foremost, I would like express my deepest thanks and appreciation to my supervisors, Dr. Lee Soon Leong from the Forest Research Institute Malaysia (FRIM) and Dr. Faridah Qamaruz Zaman and Associate Professor Dr. Faridah Hanum Ibrahim from the Universiti Putra Malaysia for their huge contribution, guidance and keen interest in my research. Their criticisms, support and valuable suggestions were most helpful throughout the process of completing this thesis.

My special thanks go to the Director General of FRIM, Dato' Dr. Abdul Razak Mohd. Ali, for granting me a Master Research Assistantship to pursue the study. I also thank my Senior Divisional Director, Dr. Daniel Baskaran Krishnapillay, and Biotechnology Programme Director, Dr. Marzalina Mansor, for their encouragement and unending support.

The Forest Departments of Kedah, Perak, Selangor, Negeri Sembilan, Johor, Pahang, Terengganu and Kelantan are acknowledged for granting me permission to access the forest reserves. The District Forest Officers and the staffs of the Renjer Offices provided assistance and logistic support during the field trips. I am indebted to Dr. Eric B. Vincent from Promega Corporation, for the use of the modified PowerStats Worksheet Templates for forensic parameters calculation.

I am particularly grateful to my Genetics Laboratory colleagues, Dr. Kevin Ng Kit Siong, Dr. Ng Chin Hong and Ms. Lee Chai Ting, who guided me and coached me throughout. I also thank Dr. Norwati Muhammad, Dr. Norwati Adnan and Ms. Norlia

Basherudin, who gave their help directly or indirectly. Special thanks go to Mariam Din, Ghazali Jaafar, Yahya Marhani, Ramli Ponyoh, Sharifah Talib, Suryani Che Seman, Nurul Hudaini Mamat and Nor Salwah Abdul Wahid for their excellent assistance in the laboratory and the field. All research group members were helpful with their information. All my colleagues, especially Ms. Sun Wan Fong, Ms. Ho Wai Mun and Mr. Brian Yap Jing Wei, gave much support and encouragement.

Last but not least, I owe my deepest appreciation and thanks to my parents, Mr. Tnah Boon Keat, Madam Low Soo Chow, Mr. Tan Seng Wah, Madam Tan Siok Gern and all the family members for their concern, care and encouragement throughout the study. To my beloved husband Kay Win, I cannot express how much his love, support and understanding have meant to me over the years and I look forward to their continuity in years to come.

I certify that an Examination Committee has met on 31<sup>st</sup> July 2007 to conduct the final examination of Tnah Lee Hong on her Master of Science thesis entitled “DNA Fingerprint Databases of Chengal (*Neobalanocarpus heimii*) for Forensic Forestry Investigations” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee are as follows:

**Siti Khadijah Daud, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Chairman)

**Tan Soon Guan, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Rozi Mohamed, PhD**

Lecturer  
Faculty of Forestry  
Universiti Putra Malaysia  
(Internal Examiner)

**Yoshihiko Tsumura, PhD**

Head of Tree Genetics Lab  
Department of Forest Genetics  
Forestry and Forest Products Research Institute  
(External Examiner)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Faridah Qamaruz Zaman, PhD**

Lecturer  
Institute of Bioscience  
Universiti Putra Malaysia  
(Chairman)

**Lee Soon Leong, PhD**

Forest Biotechnology Division  
Forest Research Institute Malaysia  
(Member)

**Faridah Hanum Ibrahim, PhD**

Associate Professor  
Faculty of Forestry  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD.**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

**TNAH LEE HONG**

Date: 13 August 2007

## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL</b>	ix
<b>DECLARATION</b>	xi
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xvi
<b>LIST OF ABBREVIATIONS</b>	xvii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>7</b>
2.1 Illegal logging	7
2.2 Tandem-repetitive DNA as molecular markers	10
2.2.1 Category of STRs	12
2.2.2 Evolution and mutation mechanisms of STRs	13
2.2.3 Theoretical mutation model of STRs	16
2.2.4 Potential function of STRs	18
2.2.5 STRs as molecular markers	19
2.3 DNA technology in forensic science	25
2.3.1 History of forensic DNA analysis	26
2.3.2 General outline of modern DNA fingerprinting	29
2.3.3 STR population databases	32
2.3.4 The product rule	34
2.3.5 The subpopulation model	36
2.3.6 Botany forensic application	38
2.4 Description of <i>Neobalanocarpus heimii</i> (King) Ashton	40
<b>3 MATERIALS AND METHODS</b>	<b>45</b>
3.1 Sample collections and plant materials	45
3.2 DNA extraction and purification	48
3.3 PCR amplification and electrophoresis conditions	49
3.4 Selection of STR loci	49
3.4.1 Specific amplification and level of polymorphism	51
3.4.2 Single-locus mode of inheritance	51
3.4.3 Presence of null alleles	51
3.4.4 Size homoplasy and variation in repeat motif	52
3.5 Statistical analyses	53
3.5.1 Intra-population STR diversity	53
3.5.2 Inter-population STR diversity	59

3.5.3	Characterization of database at the regional level	61
3.5.4	Characterization of database at the Peninsular Malaysia level	62
3.5.5	Forensic parameters	62
3.6	Selection of statistical model and conservativeness of the databases	64
<b>4</b>	<b>RESULTS</b>	<b>66</b>
4.1	Selection of STRs	66
4.1.1	Specific amplifications and polymorphisms	66
4.1.2	Single-locus modes of inheritance	68
4.1.3	Presence of null alleles	68
4.1.4	Variations in repeat motif and size homoplasy	68
4.2	Characterization of database at the population level	73
4.3	Partition of populations according to region	79
4.4	Characterization of database at the regional level	84
4.5	Characterization of database at the Peninsular Malaysia level	90
4.6	Selection of statistical model	92
4.7	Conservativeness of the databases	94
<b>5</b>	<b>DISCUSSION</b>	<b>95</b>
5.1	Selection of STRs	95
5.2	Intra-population STR diversity	102
5.3	Inter-population STR diversity	105
5.4	Hardy-Weinberg proposition and independence of loci	108
5.5	Which model to use?	110
5.6	Conservativeness of the regional and Peninsular Malaysian database	113
<b>6</b>	<b>CONCLUSION</b>	<b>116</b>
	<b>REFERENCES</b>	<b>122</b>
	<b>APPENDICES</b>	<b>146</b>
	<b>BIODATA OF THE AUTHOR</b>	<b>192</b>

## LIST OF TABLES

Table		Page
3.1	Summary of the populations, forest reserves, accession symbols, locations, numbers of samples ( $n$ ) and states of origin of the samples genotyped in this study.	46
3.2	Six native STRs of <i>Neobalanocarpus heimii</i> (Iwata <i>et al.</i> 2000) and 45 STRs developed for <i>Hopea bilitonensis</i> (Lee <i>et al.</i> 2004a), <i>Shorea leprosula</i> (Lee <i>et al.</i> 2004b), <i>S. lumutensis</i> (Lee <i>et al.</i> 2006b) and <i>S. curtisii</i> (Ujino <i>et al.</i> 1998) used for primer screening in <i>N. heimii</i> .	50
4.1	The estimated null allele frequencies of 16 STRs of <i>Neobalanocarpus heimii</i> in the Pasoh Forest Reserve using three algorithms described by Chakraborty <i>et al.</i> (1992), Brookfield (1996) and Oosterhout <i>et al.</i> (2004).	69
4.2	Allele frequencies and statistical parameters for 12 STRs ( <i>Nhe004</i> , <i>Nhe005</i> , <i>Nhe011</i> , <i>Nhe015</i> , <i>Nhe018</i> , <i>Hbi161</i> , <i>Sle392</i> , <i>Sle605</i> , <i>Slu044a</i> , <i>Shc03</i> , <i>Shc04</i> and <i>Shc07</i> ) based on 252 individuals of <i>Neobalanocarpus heimii</i> in the Pasoh Forest Reserve.	74
4.3	Fisher exact test for linkage equilibrium in <i>Neobalanocarpus heimii</i> across 12 STRs in Pasoh Forest Reserve ( $n = 252$ ), Region A ( $n = 302$ ), Region B ( $n = 597$ ), Region C ( $n = 182$ ) and Peninsular Malaysia ( $n = 1081$ ).	76
4.4	Estimated pedigree relationships for all pairs of 252 individuals of <i>Neobalanocarpus heimii</i> in the Pasoh Forest Reserve based on 12 STRs using ML-Relate (Kalinowski <i>et al.</i> 2006).	80
4.5	Inbreeding coefficient ( $f$ ) of 12 STRs based on 252 individuals of <i>Neobalanocarpus heimii</i> in the Pasoh Forest Reserve.	80
4.6	Allele frequencies and statistical parameters for 12 STRs ( <i>Nhe004</i> , <i>Nhe005</i> , <i>Nhe011</i> , <i>Nhe015</i> , <i>Nhe018</i> , <i>Hbi161</i> , <i>Sle392</i> , <i>Sle605</i> , <i>Slu044a</i> , <i>Shc03</i> , <i>Shc04</i> and <i>Shc07</i> ) based on 302 individuals of <i>Neobalanocarpus heimii</i> in Region A.	85



- 4.7 Allele frequencies and statistical parameters for 12 STRs (*Nhe004*, *Nhe005*, *Nhe011*, *Nhe015*, *Nhe018*, *Hbi161*, *Sle392*, *Sle605*, *Slu044a*, *Shc03*, *Shc04* and *Shc07*) based on 597 individuals of *Neobalanocarpus heimii* in Region B. 86
- 4.8 Allele frequencies and statistical parameters for 12 STRs (*Nhe004*, *Nhe005*, *Nhe011*, *Nhe015*, *Nhe018*, *Hbi161*, *Sle392*, *Sle605*, *Slu044a*, *Shc03*, *Shc04* and *Shc07*) based on 182 individuals of *Neobalanocarpus heimii* in Region C. 87
- 4.9 Coancestry coefficient ( $\theta$ ) and inbreeding coefficient ( $f$ ) of *Neobalanocarpus heimii* calculated for Regions A, B, C and Peninsular Malaysia. 89
- 4.10 Allele frequencies and statistical parameters for 12 STRs (*Nhe004*, *Nhe005*, *Nhe011*, *Nhe015*, *Nhe018*, *Hbi161*, *Sle392*, *Sle605*, *Slu044a*, *Shc03*, *Shc04* and *Shc07*) based on 1081 individuals of *Neobalanocarpus heimii* in Peninsular Malaysia. 91
- 4.11 Results of database's conservativeness demonstrating the effects of using various models (model under Hardy-Weinberg and linkage equilibrium, subpopulation model and subpopulation-cum-inbreeding model) to estimate genotype match probability for 235 individuals of *Neobalanocarpus heimii* based on the Pasoh, Region B and Peninsular Malaysian databases. 93

## LIST OF FIGURES

Figure		Page
2.1	Overview of the biology, technology and genetics of DNA fingerprinting using STR markers (Source: Butler 2005).	31
2.2	Morphological characteristics of <i>Neobalanocarpus heimii</i> .	41
3.1	Locations of 30 populations of <i>Neobalanocarpus heimii</i> in 27 forest reserves of Peninsular Malaysia.	47
4.1	Qualitative observations showing each progeny to possess at least one maternal allele to support the postulation of a single-locus mode of inheritance in <i>Slu044a</i> (A) but not in <i>Hbi329</i> (B).	67
4.2	Multiple alignments of partial sequences of 16 STRs in <i>Neobalanocarpus heimii</i> (A-P).	70
4.3	Neighbour-joining tree of 252 individuals of <i>Neobalanocarpus heimii</i> in the Pasoh Forest Reserve performed using shared allele distances ( $D_{SA}$ ; Jin and Chakraborty 1993) based on proportion of shared alleles from 12 STRs.	77
4.4	Distograms of estimated number of alleles in common based on 12 STRs for <i>Neobalanocarpus heimii</i> within the Pasoh 50-ha plot.	78
4.5	Model-based ancestry for each region.	81
4.6	Neighbour-joining analysis based on $D_C$ distances (Cavalli-Sforza and Edwards 1967) among 30 populations of <i>Neobalanocarpus heimii</i> in Peninsular Malaysia.	82
4.7	The distribution of <i>Neobalanocarpus heimii</i> in Peninsular Malaysia partitioned into three different regions (Regions A, B and C) according to the clustering methods quantified using STRUCTURE (Pritchard <i>et al.</i> 2000) and Chord distance, $D_C$ (Cavalli-Sforza and Edwards 1967).	83

## LIST OF ABBREVIATIONS

AABB	American Association of Blood Banks
AFP	Asia Forest Partnership
AFLP	Amplified fragment length polymorphism
bp	Base pair
CODIS	Combined DNA Index System
CTAB	Hexadecyltrimethyl-ammonium bromide
DNA	Deoxyribonucleic acid
dbh	Diameter at breast height
dNTP	2'-deoxynucleoside 5'-triphosphate
EDNAP	European DNA Profiling Group
EDTA	Diaminoethanetetra-acetic acid
FAO	Food and Agricultural Organization
FBI	Federal Bureau of Investigations
FFD	Federal Forest Department
FLEG	Forest Law Enforcement and Governance
FR	Forest Reserve
FRIM	Forest Research Institute Malaysia
GDA	Genetic Data Analysis
GSM	Generalized stepwise model
HWE	Hardy-Weinberg equilibrium
IAM	Infinite allele model
IUCN	International Union for Conservation of Nature and Natural Resources
KAM	K-allele model

kb	Kilobase pair
LE	Linkage equilibrium
LEI	Indonesia Ecolabelling Institute
Mb	Megabase pair
MgCl <sub>2</sub>	Magnesium chloride
min	Minute
MLP	Multi-locus probe
MP	Match probability
MTC	Malaysian Timber Council
MTCC	Malaysian Timber Certification Council
myr	Million years
NaCl	Sodium chloride
NDNAD	National DNA Database
NH <sub>4</sub> OAc	Ammonium acetate
NRC	National Research Council
PCR	Polymerase chain reaction
PD	Power of discrimination
PIC	Polymorphic information content
PVP-40	Polyvinylpyrrolidone-40
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
sec	Second
SGM	Second-generation multiplex
SGS	Spatial Genetic Structure
SLP	Single-locus probe

SMM	Stepwise mutation model
SSR	Simple sequence repeat
STR	Short tandem repeat
TAE	Tris-acetate EDTA
TBE	Tris-borate EDTA
TE	Tris-EDTA
TNC	The Nature Conservancy
Tris	Trishydroxymethylaminomethane
TPM	Two phase model
UK	United Kingdom
US	United States
VU	Vulnerable
WWF	World Wildlife Fund

## **CHAPTER 1**

### **INTRODUCTION**

The tropical forest coverage in Asia has been declining at an exponential rate for several decades. Large areas of forest are either being lost to conversion for agriculture or degraded through poor logging practices without regard to sustainability and biodiversity (AFP 2005). One of the major threats to the sustainability of tropical forests is uncontrolled illegal logging. Illegal logging can be defined as an activity when forest products are bought or sold in violation or circumvention of national or subnational laws relating to harvesting, transportation and processing (White and Sarshar 2004). These include large- and small-scale timber thefts, transfer pricing, breaching of tax rules, and illegal aspects of timber sourcing and circumvention of agreements through bribery or deception. The vast extent of the illegal logging could contribute to increased poverty and land/social conflicts, causes significant losses of tax revenues, and more importantly, poses a significant threat to the sustainability of forest ecosystems (AFP 2005).

In Peninsular Malaysia, forest offences have been classified into three main categories (MTC 2004): (1) logging without license, logging outside licensed area and unauthorized construction of infrastructure and forest roads; (2) encroachment of forest reserves for agricultural activities and settlement; and (3) felling of unmarked trees, cutting trees below the limit, unlicensed workers, contractors with no valid sublicense, unregistered machinery plus other breaches of rules and regulations within and outside the forest reserve. While the constituent of illegal logging is not

clearly defined, the Malaysian Timber Council (MTC) reported a total of 1811 cases of illegal logging from 1987 to 2003 (MTC 2004).

For the case of logging without license, foresters at the moment have to depend on wood anatomical evidences to link the suspected timber thefts to the source trees. But this is inadequate as identification can only be done on the group of trees and not to the species and individual levels. Thus, in order to establish a linkage between the evidentiary sample and the source, utilization of DNA fingerprinting (synonymous to DNA typing or DNA profiling; Jackson and Jackson 2004) evidences by comparing the DNA profiles of logs with those of the stumps from which the timber is believed to have originated might provide a solution to legal cases against illegal loggers.

Forensic science can be defined as the study relating to the application of science to determine evidentiary value of items found during criminal investigation. DNA fingerprinting has long been used in humans for legal proceedings to prove guilt or innocence, resolve unestablished paternity, identify remains of missing persons or victims of mass disasters and establish citizenship by proving blood relationships in immigration laws (Butler 2005). Recently DNA fingerprinting has also been applied for the identification of animals where the issues of endangered species and breeding are significant (Hansen *et al.* 2001; Manel *et al.* 2002; Withler *et al.* 2004; Kobilinsky *et al.* 2005). In forensic botany, samples of plant materials are used to solve criminal and civil cases, and plant DNA fingerprints have been used as evidence to link the individual on whom the plant material was found to a crime scene (Yoon 1993; Siver *et al.* 1994; Congiu *et al.* 2000). Nonetheless, a comprehensive forensic procedure for plants has yet to be developed. This might be

due to the lack of awareness by evidence collection teams, difficulty in routinely identifying trace material by traditional morphological methods using whole-plant identification or by botanical experts, and significant resources required to construct population databases for the large number of plant species that may be encountered in forensic casework (Miller Coyle *et al.* 2001).

Some progress has already been made towards the development of DNA fingerprinting methods for discriminating among varieties, populations and individuals of plants such as strawberry (Congiu *et al.* 2000), *Cannabis sativa* (Gilmore *et al.* 2003), *Brachythecium albicans*, (Korpelainen and Virtanen 2003), *Ceratodon purpureus* (Korpelainen and Virtanen 2003), *Albies alba* (Ziegenhagen *et al.* 2003) and *Acer rubrum* (Bless *et al.* 2006). These DNA-based methods included random amplified fragment length polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP) and short tandem repeat (STR), which offer the potential to aid the forensic community by enabling identification of botanical samples and the determination of the provenance of seized samples (Yoon 1993; Becker *et al.* 1995; Gilmore *et al.* 2003).

STRs are short (1–6 bp in length) tandemly repeated DNA sequences. They comprise simple mononucleotide to hexanucleotide repeats, varying from a few tens of bases up to typically one hundred. These regions occur frequently and are spread randomly throughout the genomes of animals and plants, and typically show extensive variation (Jarne and Lagonda 1996). The number of tandemly repeated units has been shown to be highly polymorphic between individuals and this is thought to be due to slippage of the DNA polymerase during the synthesis and mismatched repair



(Levinson and Gutman 1987). STRs have become popular DNA markers because they are amenable to polymerase chain reaction (PCR) amplification and are highly polymorphic among individuals, which make them suitable for DNA fingerprinting (Butler 2005).

In forensic forestry investigations, in order to link the evidentiary sample (logs) and the source (stumps), two evidences need to be established, i.e. identification and individualization (Miller Coyle *et al.* 2003). Identification of a timber species can be done using wood anatomical examination for macroscopic and microscopic characters (Soerianegara and Lemmens 1994). Individualization of a sample can be carried out using DNA markers to establish a linkage between the evidentiary sample and the source (Miller Coyle *et al.* 2003). STRs are often believed to be more superior to RAPD and AFLP for individual identification and individualization, because in principle, alleles and genotypes can be unambiguously assigned, and primer sequences can be easily distributed and shared among different laboratories (Weising *et al.* 2005).

Population genetics and forensic biology are inextricable linked disciplines. In DNA testimony, it is necessary to provide an estimate of the weight of the evidence. However, since it is not possible to generate the DNA profile of every individual in a particular population, this weight needs to be assigned by applying population genetic principles and models (Buckleton *et al.* 2006). There are three possible outcomes of a DNA test: no match, inconclusive, or match between samples examined. However, only the third outcome requires statistics to answer the following question: are they from the same individual or is there something else out