

DNA PROFILING TECHNIQUES FOR TIMBER TRACKING



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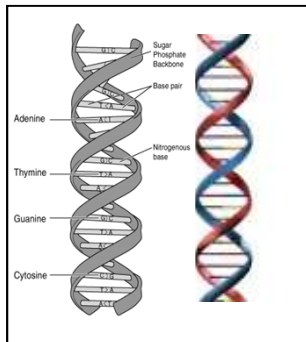
Illegal logging poses a significant threat to the sustainability of tropical forest ecosystems

Presently



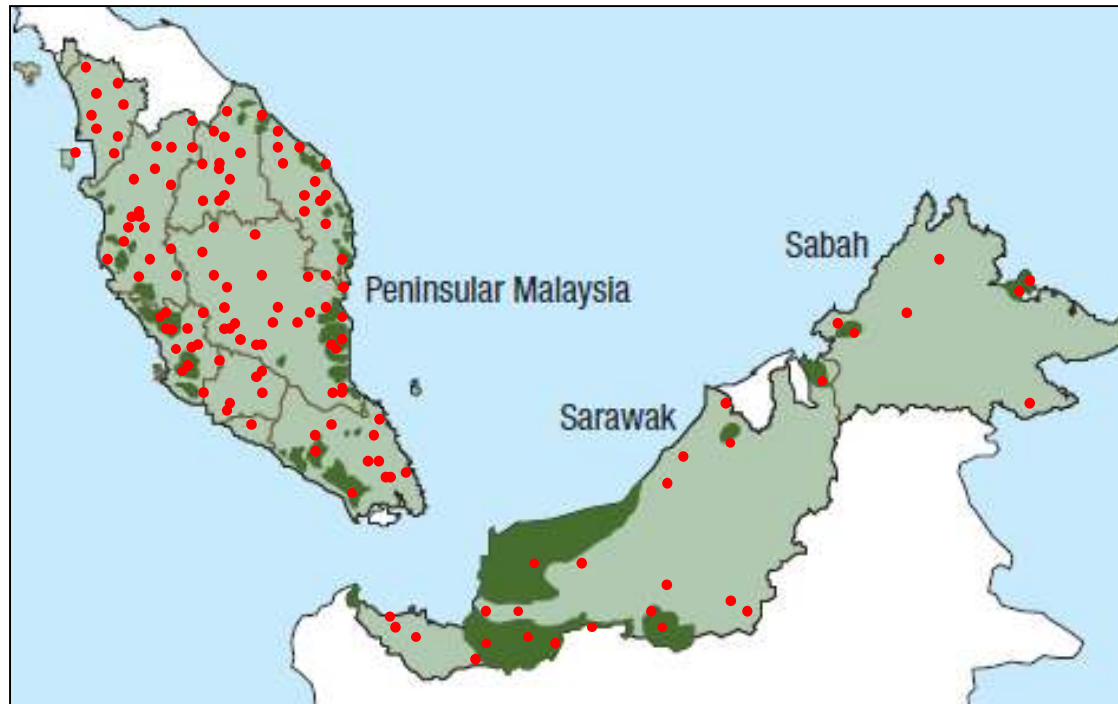
But this is inadequate as identification can only be done on a group of trees and not to the levels of SPECIES, POPULATION and INDIVIDUAL

Hence



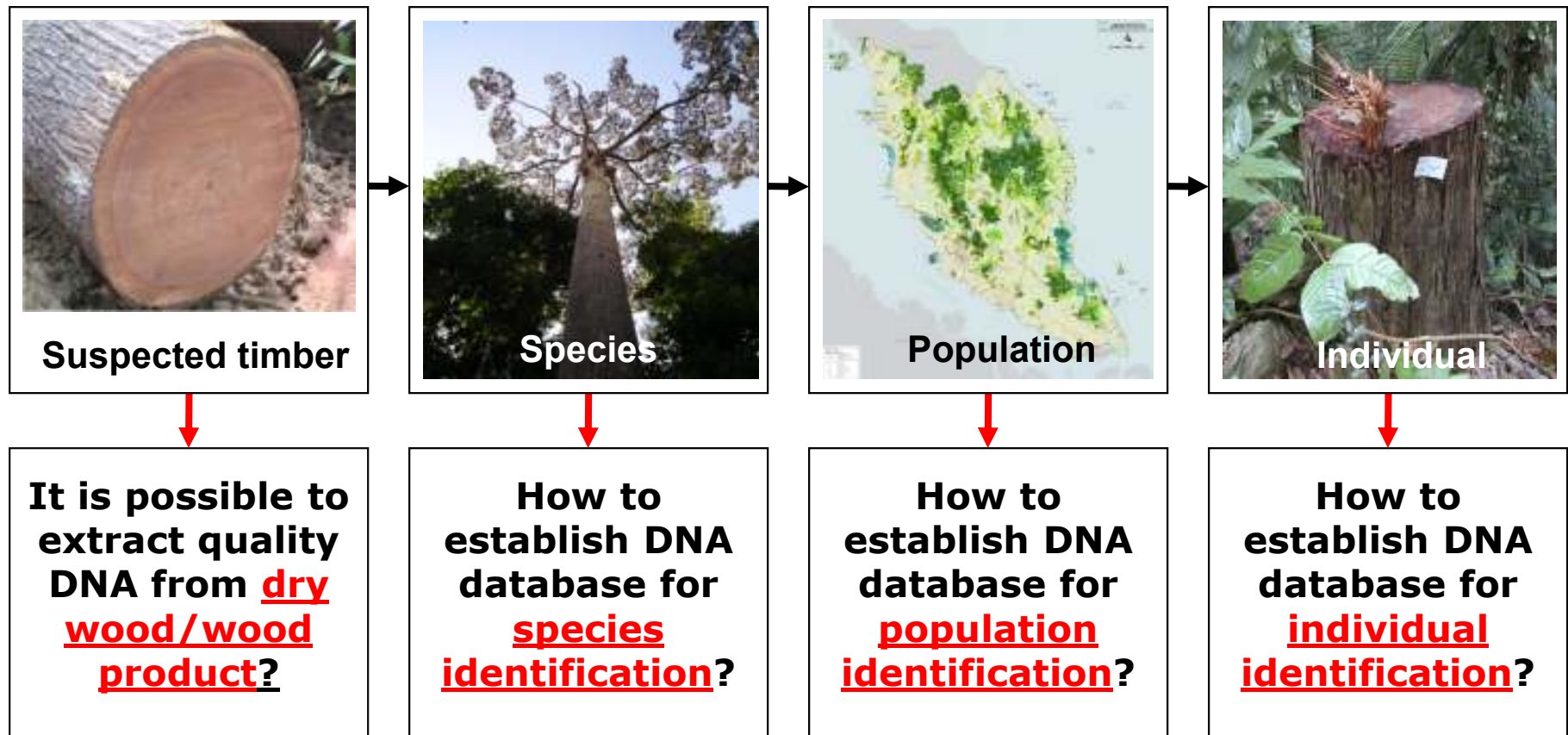
At FRIM, since 2007, by using DNA approach, we have developed DNA profiling databases of four important tropical timber species for **TIMBER TRACKING**

These four important timber species are:



1	- <i>Neobalanocarpus heimii</i>	30 pops (PM)
2	- <i>Gonystylus bancanus</i>	17 pops (PM, Sab & Sar)
3	- <i>Koompassia malaccensis</i>	64 pops (PM, Sab & Sar)
4	- <i>Shorea platyclados</i>	30 pops (PM, Sab & Sar)

How to utilise DNA markers as an instrument for timber tracking?



Neobalanocarpus heimii (Chengal)

We have successfully developed a DNA-based timber tracking system for an importance timber in Malaysia



Journal of Tropical Forest Science 22(2): 214-226 (2010)

Truh et al.

HIGHLY VARIABLE STR MARKERS OF *NEOBALANOCARPUS HEIMI* (DIPTEROCARPACEAE) FOR FORENSIC DNA PROFILING

LH Truh¹, SL Lee^{1,2}, KKS Ng¹, QZ Faridah³ & I Faridah-Hanum³

Forest Ecology and Management 258 (2010) 1420–1430

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Forensic DNA profiling of tropical timber species in Peninsular Malaysia

Lee Hong Truh¹, Soon Leong Lee^{1,2}, Kevin Kit Siong Ng¹, Qammar-Zaman Faridah³, Ibrahim Faridah-Hanum³

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Geographical traceability of an important tropical timber (*Neobalanocarpus heimii*) inferred from chloroplast DNA

Lee Hong Truh¹, Soon Leong Lee^{1,2}, Kevin K.S. Ng¹, Nooki Tani³, Subha Bhassu⁴, Rofina Yasmin Othman⁵

Wood Sci Technol

DOI 10.1007/s00226-011-0447-6

ORIGINAL

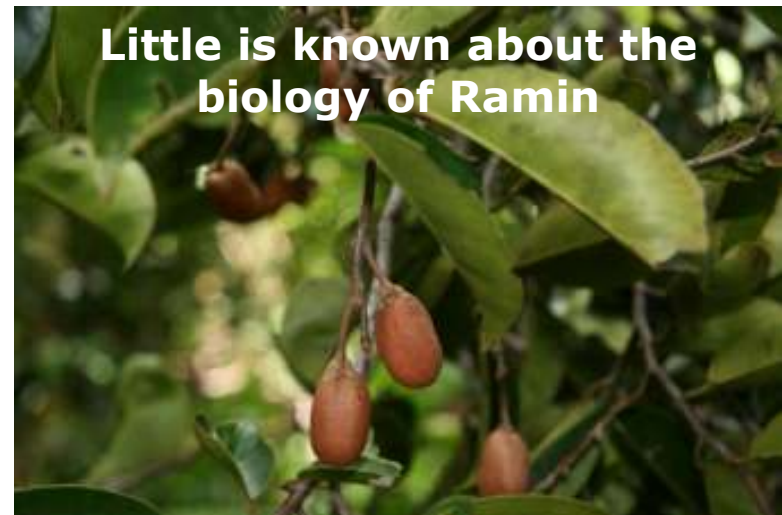
DNA extraction from dry wood of *Neobalanocarpus heimii* (Dipterocarpaceae) for forensic DNA profiling and timber tracking

Lee Hong Truh · Soon Leong Lee · Kevin Kit Siong Ng · Subha Bhassu · Rofina Yasmin Othman

***Gonystylus bancanus* (Ramin)**



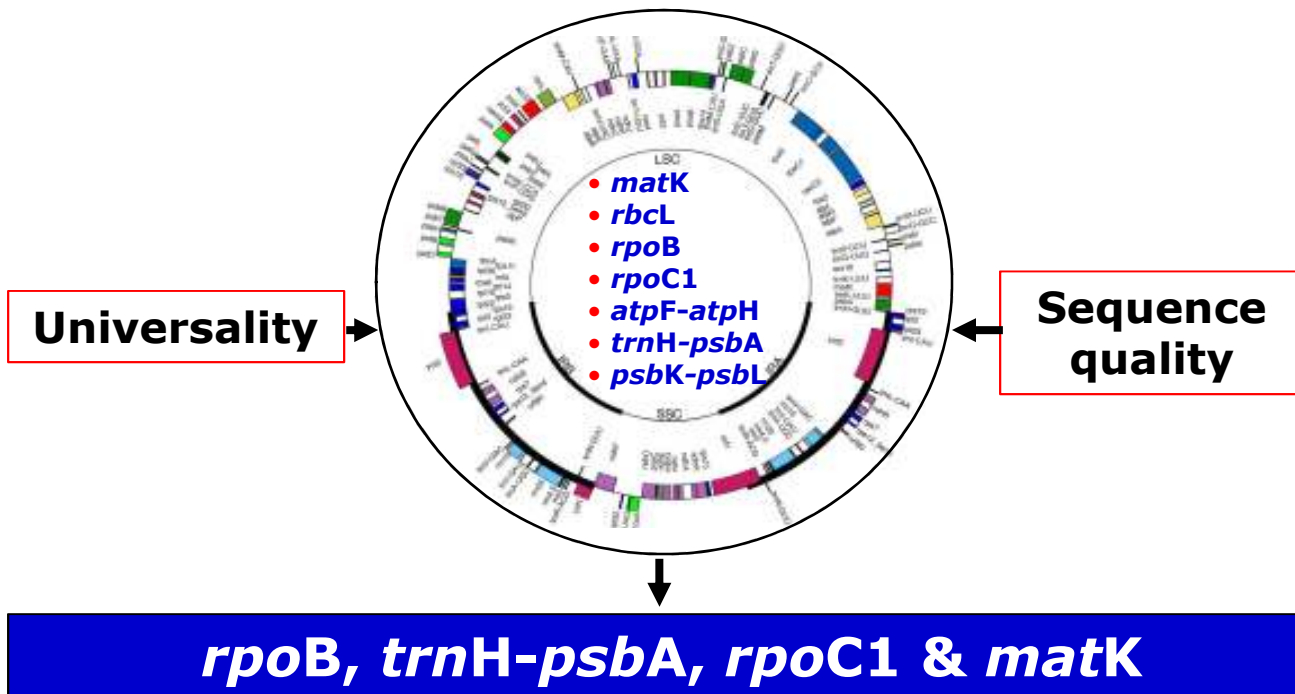
Valuable timber



How to establish DNA database for species identification?

DNA barcodes

408 Malaysia timber species are known in the international market



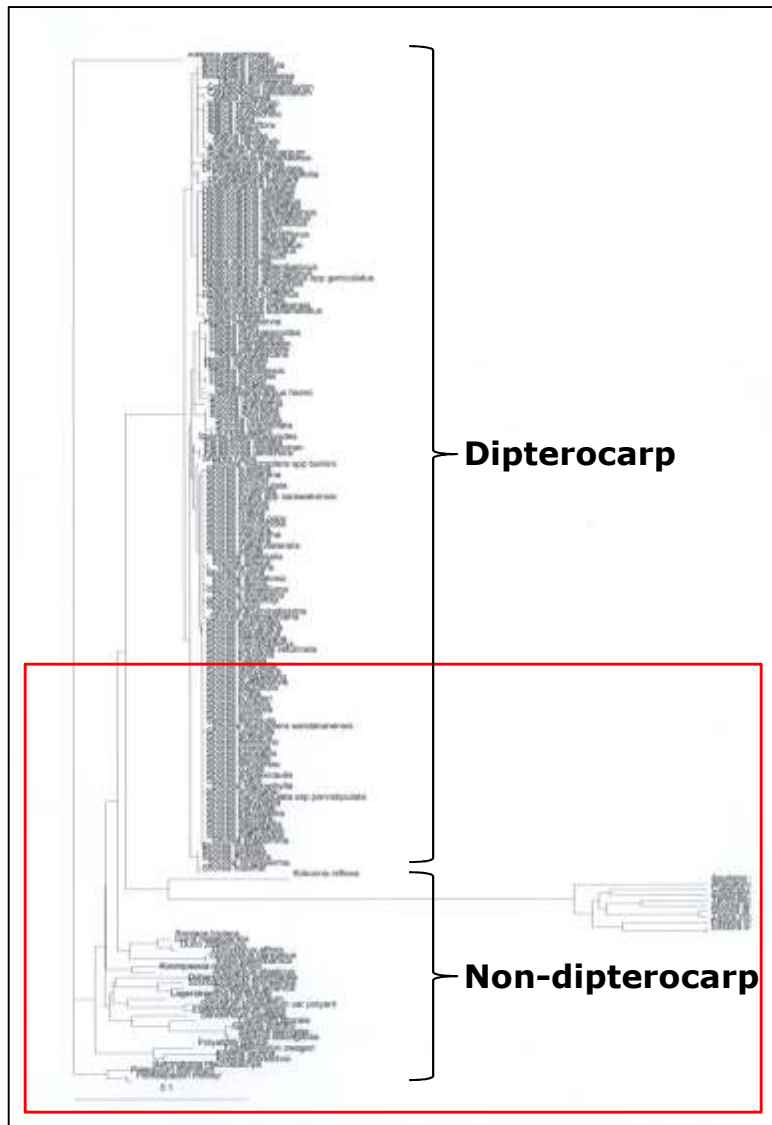
rpoB, trnH-psbA, rpoC1 & matK

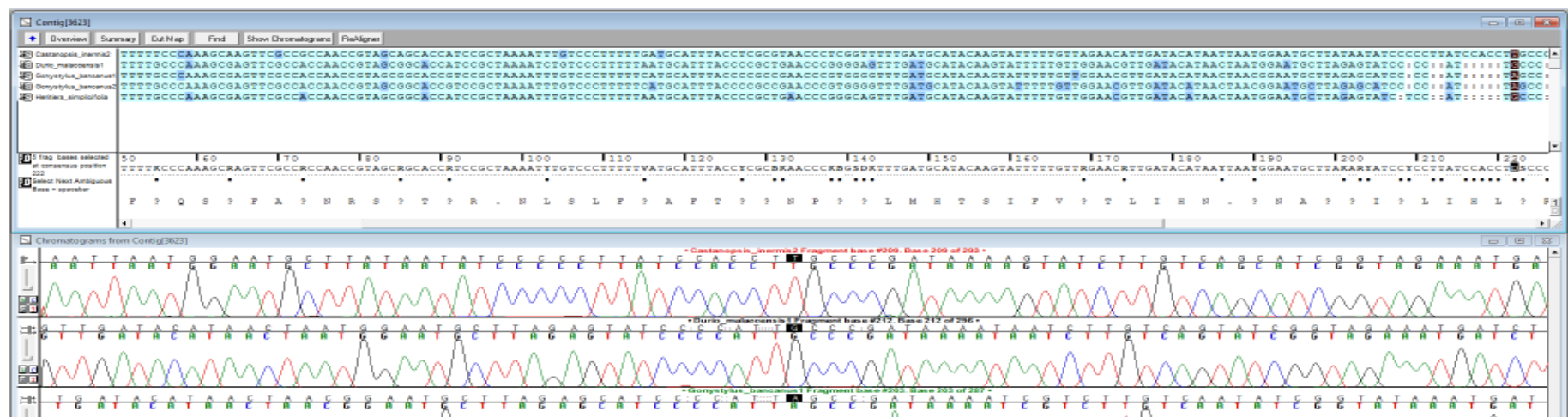
204 major timber species from 26 families

Anacardiaceae, Annonaceae, Apocynaceae, Bombacaceae, Burseraceae, Celastraceae, Combretaceae, Dipterocarpaceae, Euphorbiaceae, Fagaceae, Guttiferae, Lauraceae, Lecythidaceae, Leguminosae, Loganiaceae, Lythraceae, Meliaceae, Moraceae, Myristicaceae, Myrtaceae, Olacaceae, Sapindaceae, Sapotaceae, Sterculiaceae, Thymelaeaceae & Tiliaceae

<i>Alstonia angustiloba</i>	<i>Dipterocarpus kerrii</i>	<i>Hopea helferi</i>	<i>Sandoricum koetjape</i>	<i>Shorea guiso</i>	<i>Shorea resinosa</i>
<i>Anisoptera costata</i>	<i>Dipterocarpus lowii</i>	<i>Hopea latifolia</i>	<i>Santiria griffithii</i>	<i>Shorea hemsleyana</i>	<i>Shorea richetia</i>
<i>Anisoptera curtisii</i>	<i>Dipterocarpus oblongifolius</i>	<i>Hopea mengerawan</i>	<i>Santiria laevigata</i>	<i>Shorea henryana</i>	<i>Shorea roxburghii</i>
<i>Anisoptera grossivenia</i>	<i>Dipterocarpus obtusifolius</i>	<i>Hopea nervosa</i>	<i>Santiria oblongifolia</i>	<i>Shorea inaequilateralis</i>	<i>Shorea rubra</i>
<i>Anisoptera laevis</i>	<i>Dipterocarpus palembanicus</i>	<i>Hopea nutans</i>	<i>Scaphium linearicarpum</i>	<i>Shorea isoptera</i>	<i>Shorea rugosa</i>
<i>Anisoptera scaphula</i>	<i>Dipterocarpus perakensis</i>	<i>Hopea odorata</i>	<i>Scorodocarpus borneensis</i>	<i>Shorea kunstleri</i>	<i>Shorea scaberrima</i>
<i>Aquilaria malaccensis</i>	<i>Dipterocarpus rigidus</i>	<i>Hopea pentanervia</i>	<i>Shorea acuminata</i>	<i>Shorea ladiana</i>	<i>Shorea superba</i>
<i>Artocarpus elasticus</i>	<i>Dipterocarpus rotundifolius</i>	<i>Hopea pubescens</i>	<i>Shorea acuminatissima</i>	<i>Shorea laevis</i>	<i>Shorea teysmanniana</i>
<i>Artocarpus lanceifolius</i>	<i>Dipterocarpus sarawakensis</i>	<i>Hopea sangal</i>	<i>Shorea agami</i>	<i>Shorea lepidota</i>	<i>Shorea uliginosa</i>
<i>Artocarpus rigidus</i>	<i>Dipterocarpus semivestitus</i>	<i>Hopea subalata</i>	<i>Shorea albida</i>	<i>Shorea leprosula</i>	<i>Shorea xanthophylla</i>
<i>Artocarpus scortechinii</i>	<i>Dipterocarpus stellatus</i>	<i>Hopea wightiana</i>	<i>Shorea almon</i>	<i>Shorea longisperma</i>	<i>Sindora coriacea</i>
<i>Azadirachta excels</i>	<i>Dipterocarpus sublamellatus</i>	<i>Instia palembanica</i>	<i>Shorea amplexicaulis</i>	<i>Shorea macrantha</i>	<i>Sindora echinocalyx</i>
<i>Barringtonia macrostachya</i>	<i>Dipterocarpus tempehes</i>	<i>Knema laurina</i>	<i>Shorea argentifolia</i>	<i>Shorea macrophylla</i>	<i>Strombosia javanica</i>
<i>Callerya atropurpurea</i>	<i>Dryobalanops aromatica</i>	<i>Knema scortechinii</i>	<i>Shorea assamica</i>	<i>Shorea macroptera</i>	<i>Syzygium cinereum</i>
<i>Canarium littorale</i>	<i>Dryobalanops beccarii</i>	<i>Kokoona reflexa</i>	<i>Shorea atrinervosa</i>	<i>Shorea materialis</i>	<i>Syzygium polyanthum</i>
<i>Canarium patentinervium</i>	<i>Dryobalanops lanceolata</i>	<i>Koompassia malaccensis</i>	<i>Shorea balanocarpoides</i>	<i>Shorea maxima</i>	<i>Terminalia calamansanai</i>
<i>Castanopsis inermis</i>	<i>Dryobalanops oblongifolia</i>	<i>Lagerstroemia floribunda</i>	<i>Shorea beccariana</i>	<i>Shorea maxwelliana</i>	<i>Terminalia catappa</i>
<i>Cotylelobium lanceolatum</i>	<i>Dryobalanops rappa</i>	<i>Lithocarpus curtisii</i>	<i>Shorea bracteolata</i>	<i>Shorea mecistopteryx</i>	<i>Terminalia phellocarpa</i>
<i>Cotylelobium melanoxylon</i>	<i>Durio graveolens</i>	<i>Lithocarpus wallichianus</i>	<i>Shorea collina</i>	<i>Shorea multiflora</i>	<i>Terminalia subspatulata</i>
<i>Crudia curtisii</i>	<i>Durio griffithii</i>	<i>Litsea elliptica</i>	<i>Shorea coriacea</i>	<i>Shorea obscura</i>	<i>Upuna borneensis</i>
<i>Dipterocarpus acutangulus</i>	<i>Durio malaccensis</i>	<i>Mangifera foetida</i>	<i>Shorea crassa</i>	<i>Shorea ochracea</i>	<i>Vatica bella</i>
<i>Dipterocarpus baudi</i>	<i>Durio zibethinus</i>	<i>Mesua ferrea</i>	<i>Shorea curtisii</i>	<i>Shorea ochrophloia</i>	<i>Vatica cinerea</i>
<i>Dipterocarpus caudatus</i>	<i>Dyera costulata</i>	<i>Neobalanocarpus heimii</i>	<i>Shorea dasyphylla</i>	<i>Shorea ovalis</i>	<i>Vatica flavida</i>
<i>Dipterocarpus chartaceus</i>	<i>Elatiospermum tapos</i>	<i>Ochanostachys amentacea</i>	<i>Shorea domatiosa</i>	<i>Shorea ovata</i>	<i>Vatica havilandii</i>
<i>Dipterocarpus confertus</i>	<i>Eusideroxylon zwageri</i>	<i>Palaquium maingayi</i>	<i>Shorea elliptica</i>	<i>Shorea palembanica</i>	<i>Vatica nitens</i>
<i>Dipterocarpus cornutus</i>	<i>Fagraea fragrans</i>	<i>Palaquium obovatum</i>	<i>Shorea exelliptica</i>	<i>Shorea palosapis</i>	<i>Vatica odorata</i>
<i>Dipterocarpus costulatus</i>	<i>Gonystylus affinis</i>	<i>Palaquium rostratum</i>	<i>Shorea faquetiana</i>	<i>Shorea parvifolia</i>	<i>Vatica pauciflora</i>
<i>Dipterocarpus crinitus</i>	<i>Gonystylus bancanus</i>	<i>Parashorea densiflora</i>	<i>Shorea falcifera</i>	<i>Shorea parvistipulata</i>	<i>Vatica scortechinii</i>
<i>Dipterocarpus elongatus</i>	<i>Heritiera simplicifolia</i>	<i>Parashorea malaanonan</i>	<i>Shorea ferruginea</i>	<i>Shorea pauciflora</i>	<i>Vatica umbonata</i>
<i>Dipterocarpus eurynchus</i>	<i>Hopea apiculata</i>	<i>Parashorea stellata</i>	<i>Shorea flaviflora</i>	<i>Shorea pilosa</i>	
<i>Dipterocarpus fagineus</i>	<i>Hopea auriculata</i>	<i>Payena obscura</i>	<i>Shorea flemmichii</i>	<i>Shorea pinanga</i>	
<i>Dipterocarpus geniculatus</i>	<i>Hopea beccariana</i>	<i>Pentace triptera</i>	<i>Shorea foxworthyi</i>	<i>Shorea platycarpa</i>	
<i>Dipterocarpus grandiflorus</i>	<i>Hopea bilitonensis</i>	<i>Pentaspadon motleyi</i>	<i>Shorea gibbosa</i>	<i>Shorea platyclados</i>	
<i>Dipterocarpus hasseltii</i>	<i>Hopea dryobalanoides</i>	<i>Polyalthia glauca</i>	<i>Shorea glauca</i>	<i>Shorea pubistyla</i>	
<i>Dipterocarpus kerrii</i>	<i>Hopea dyeri</i>	<i>Pometia pinnata</i>	<i>Shorea gratissima</i>	<i>Shorea quadrinervis</i>	
	<i>Hopea glaucescens</i>				

Chloroplast *rpoB* region (321bp)

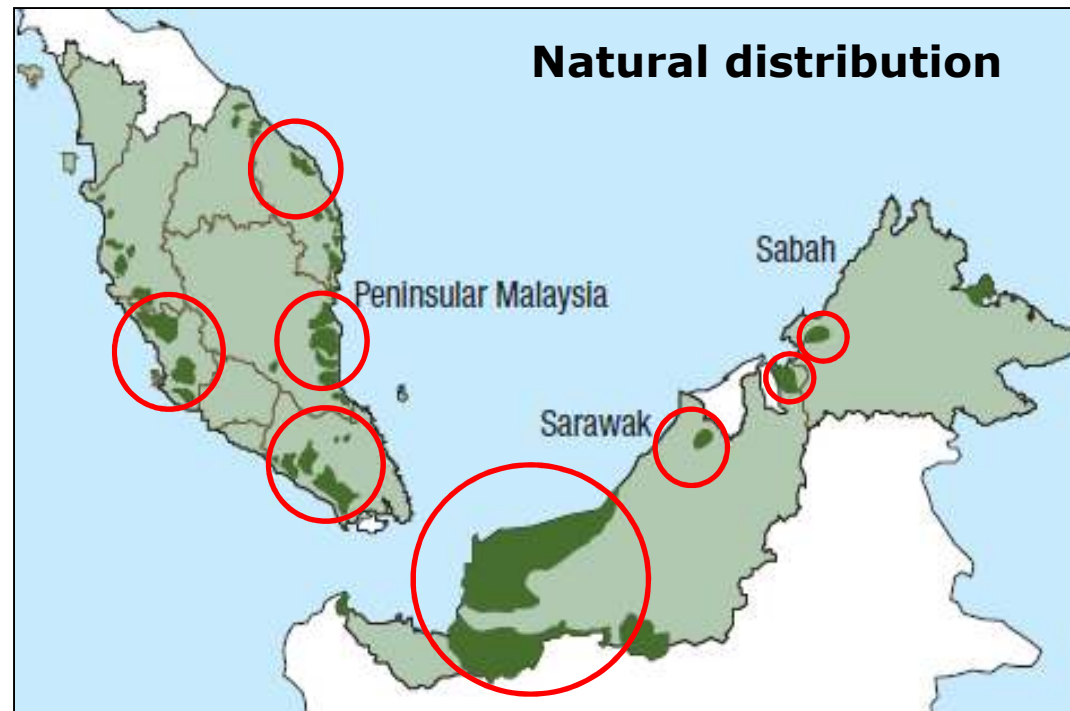




No.	Species	Sequence
35	<i>Dipterocarpus_rotundifolius</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
36	<i>Dipterocarpus_sarawakensis</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
37	<i>Dipterocarpus_stellatus</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
38	<i>Dipterocarpus_sublamellatus</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
39	<i>Dipterocarpus_tampahes</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
40	<i>Dryobalanops_beccarii</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
41	<i>Dryobalanops_lanceolata</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
42	<i>Dryobalanops_oblongifolia</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
43	<i>Dryobalanops_rappa</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
44	<i>Durio_malaccensis</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
45	<i>Durio_zibethinus</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
46	<i>Dysera_costulata</i>	CAACGCTCCTAATGAGTACTGCATCTTCAGAATTGTAACTTCCATGGCATATAAGTACTAATACGTTTTTCCCAAGAAAGTTCCACCAACCGTAGCAGCAGCGTCCGCTAAAAATTTGCTCTTTTAAATGTATTTACCC
47	<i>Elaternospermum_tapos</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
48	<i>Eusideroxyylon_zwageri</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
49	<i>Gonyostylus_affinis</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
50	<i>Gonyostylus_bancanus</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
51	<i>Hopea_apiculata</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
52	<i>Hopea_auriculata</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
53	<i>Hopea_beccariana</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
54	<i>Hopea_billitonensis</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
55	<i>Hopea_dryobalanoides</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
56	<i>Hopea_dyeri</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
57	<i>Hopea_glaucescens</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA
58	<i>Hopea_helferi</i>	GCTCTAGATTCAAGGG-T-TCCGCTGATAGCCGAACACGAGGGGAAAAATCATTATACCGGATAGTACGAAAAATCATTTATCGGGCAATGGGGATACTCTAAGCATTCTTTAGTTATGTATCAACGTTCTAACAAAAAATCTTGTA

Blast test with known sample managed to identify *G. bancanus* with 95% confident level

How to establish DNA database for population & individual identification?





Belara (Terengganu)

Resak (Pahang)





Lingga (Sarawak)



Lingga (Sarawak)

Klias (Sabah)



Loagan Bunut (Sarawak)





Sedilu (Sarawak)

Pekan FR (Pahang)



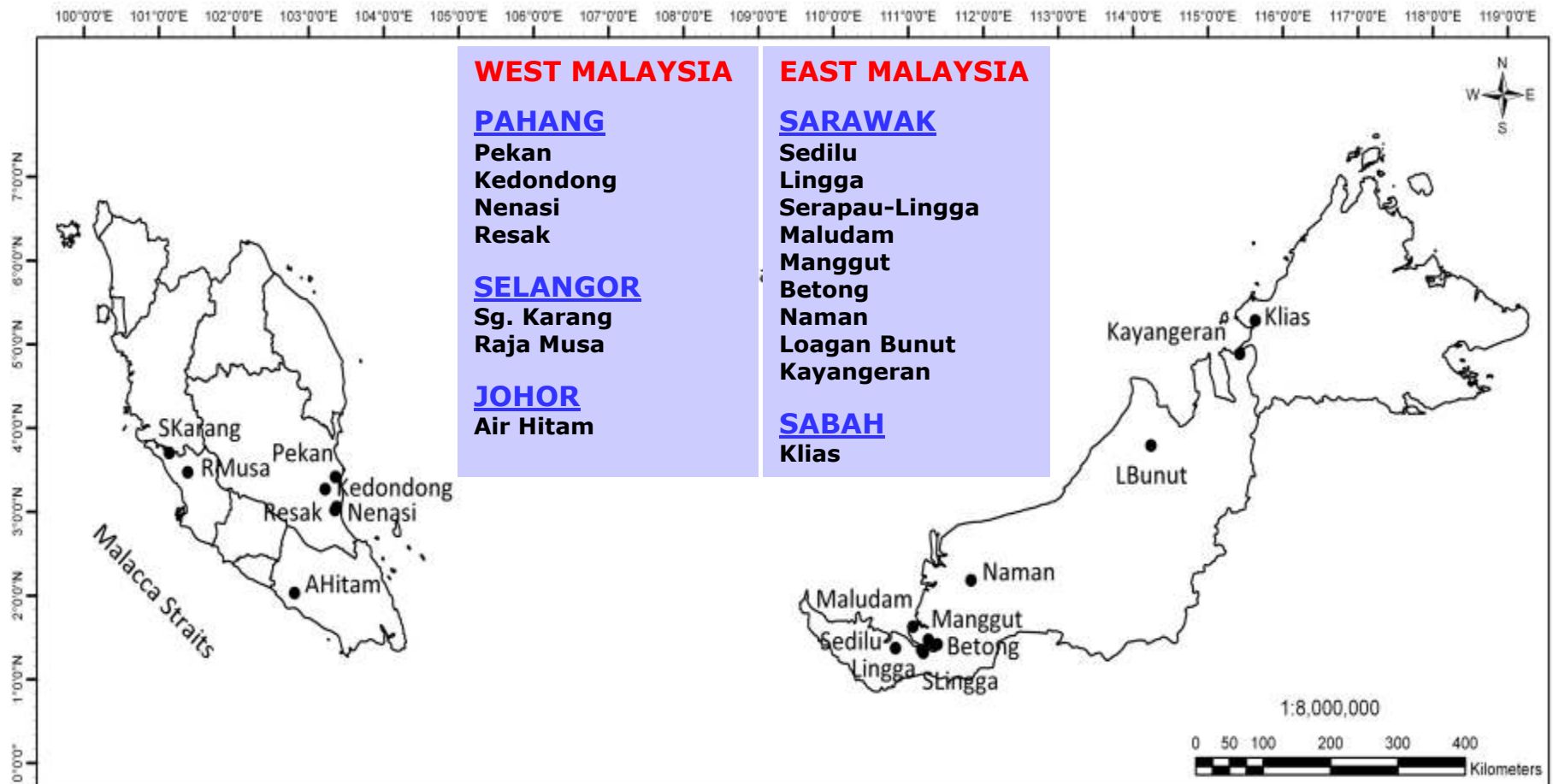


Ayer Hitam FR (Johor)



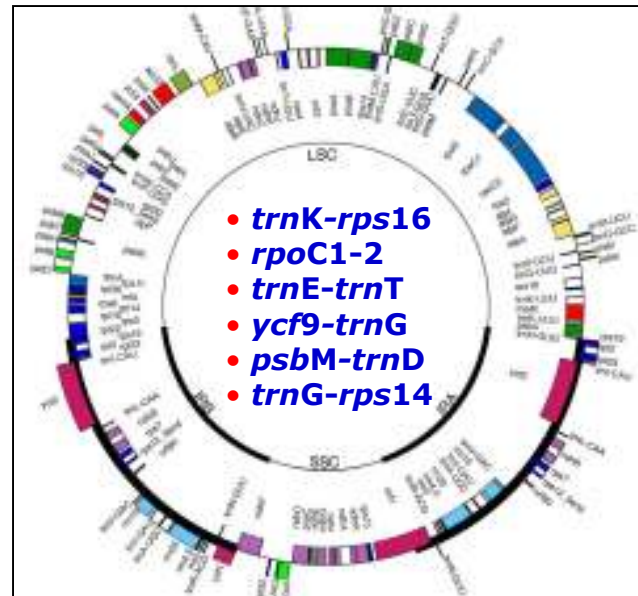
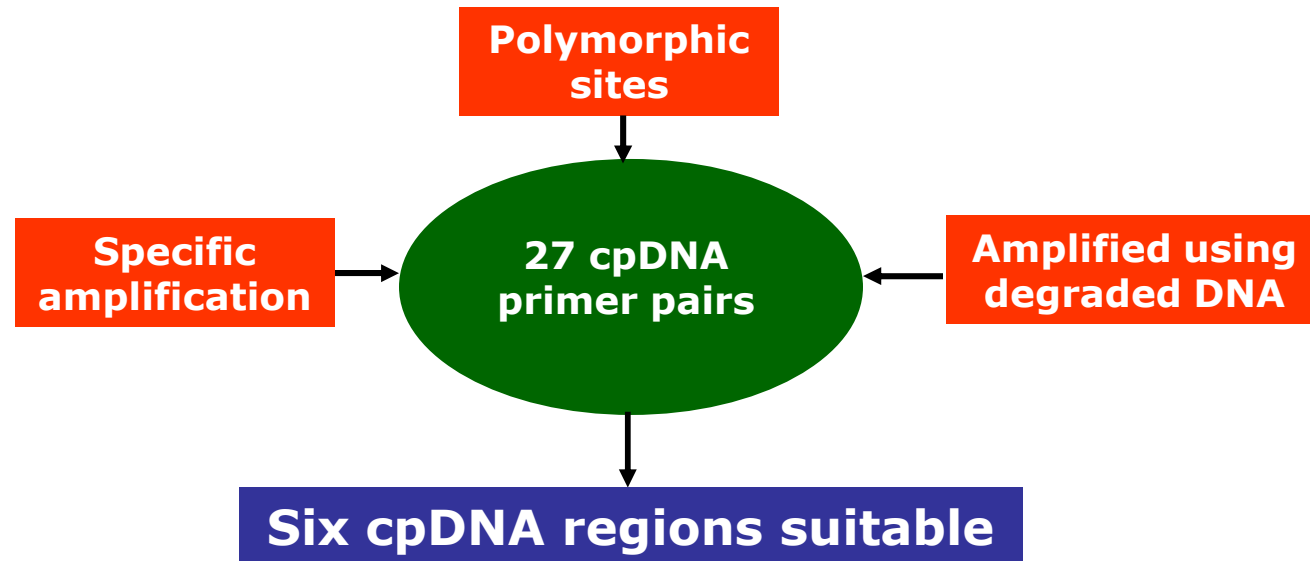
Sg Karang (Selangor)

Samples collected



747 samples were collected from 17 forest reserves throughout Malaysia

DNA database for population identification



DNA sequencing of these six regions over the 17 populations detected 24 polymorphic sites either due to indels (insertion/deletion) or base substitutions

POPULATION IDENTIFICATION DATABASE OF <i>GONYSTYLUS BANCANUS</i>																
No	Population	Individual	Haplotype	tmK-rps16						rpoC1-2			tmE-T			
				82	87	202	409	487-491	492-497	510-514	653	200	201-20	206-209	264-268	
1	Betong	BE003	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
2	Betong	BE004	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
3	Betong	BE006	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
4	Betong	BE008	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
5	Betong	BE009	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
6	Betong	BE012	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
7	Betong	BE014	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
8	Betong	BE018	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
9	Betong	BE020	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
10	Betong	BE022	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
11	Betong	BE025	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
12	Betong	BE026	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
13	Betong	BE030	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
14	Betong	BE032	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
15	Betong	BE033	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
16	Betong	BE037	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
17	Kayangeran	KG002	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
18	Kayangeran	KG003	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
19	Kayangeran	KG007	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
20	Kayangeran	KG008	P3	C	A	A	C	ATAAGA	AAATA	G	A	ATAT	
21	Kayangeran	KG012	P9	C	A	A	C	ATAAGA	G	A	ATAT	
22	Kayangeran	KG014	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
23	Kayangeran	KG016	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
24	Kayangeran	KG018	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
25	Kayangeran	KG019	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
26	Kayangeran	KG021	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
27	Kayangeran	KG023	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	
28	Kayangeran	KG025	P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT	

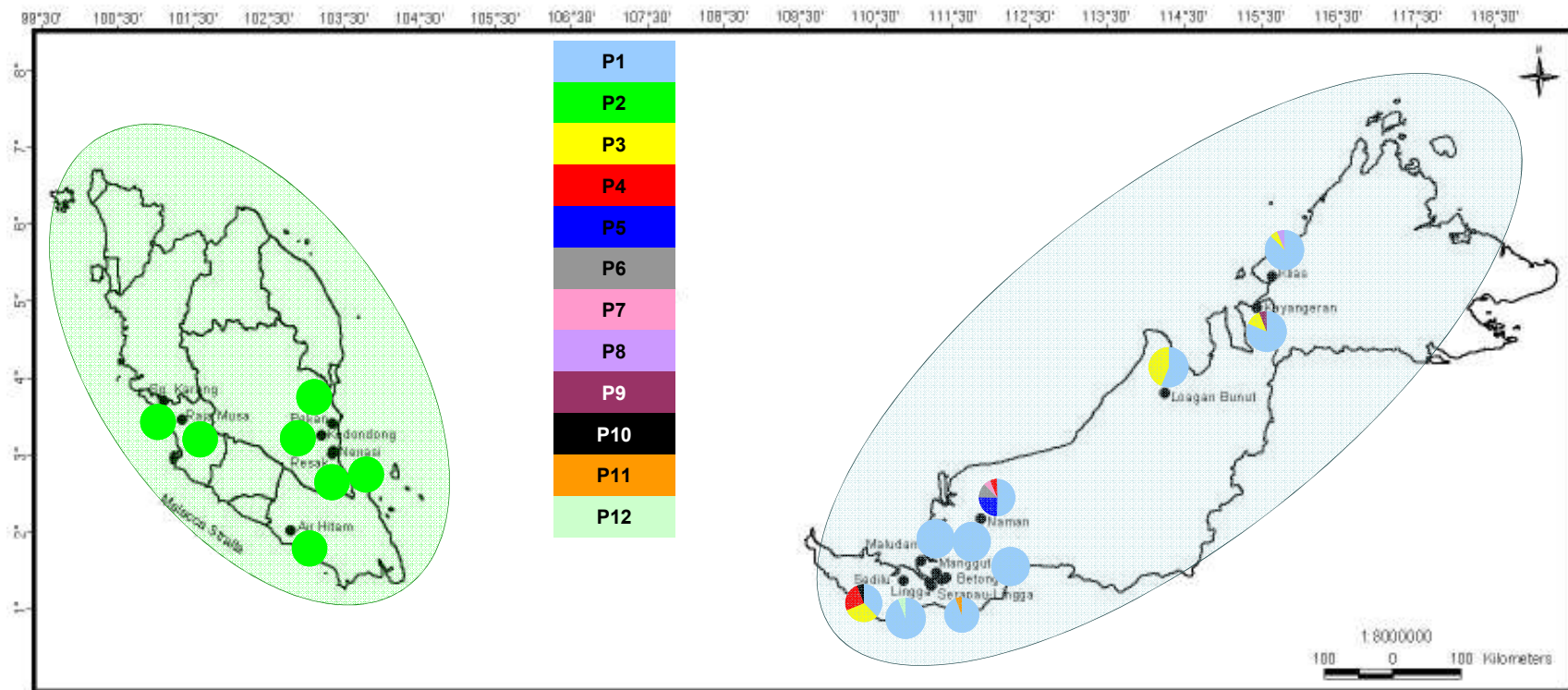
The 24 polymorphic sites generate a total of 12 haplotypes in the population database

POPULATION IDENTIFICATION DATABASE OF *GONYSTYLUS BANCANUS*

Haplotype	<i>trnK-rps16</i>					<i>rpoC1-2</i>		<i>trnE-T</i>				
	82	87	202	409	487-491	492-497	510-514	653	200	201-205	206-209	264-268
P1	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT
P2	C	A	A	C	ATAAGA	AAATA	T	A	ATAT
P3	C	A	A	C	ATAAGA	AAATA	G	A	ATAT
P4	T	G	A	C	TTATT	ATAAGA	AAATA	G	A	ATAT	GTGAT
P5	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT
P6	C	A	A	C	TCTTAT	G	A	ATAT	GTGAT
P7	C	A	A	C	ATAAGA	G	A	ATAT	GTGAT
P8	C	A	A	C	TTATT	TCTTAT	G	A	ATAT
P9	C	A	A	C	ATAAGA	G	A	ATAT
P10	C	A	A	C	ATAAGA	G	A	ATAT
P11	T	G	A	C	ATAAGA	AAATA	G	A	GAATA	ATAT	GTGAT
P12	C	A	C	T	TCTTAT	G	:	GTGAT

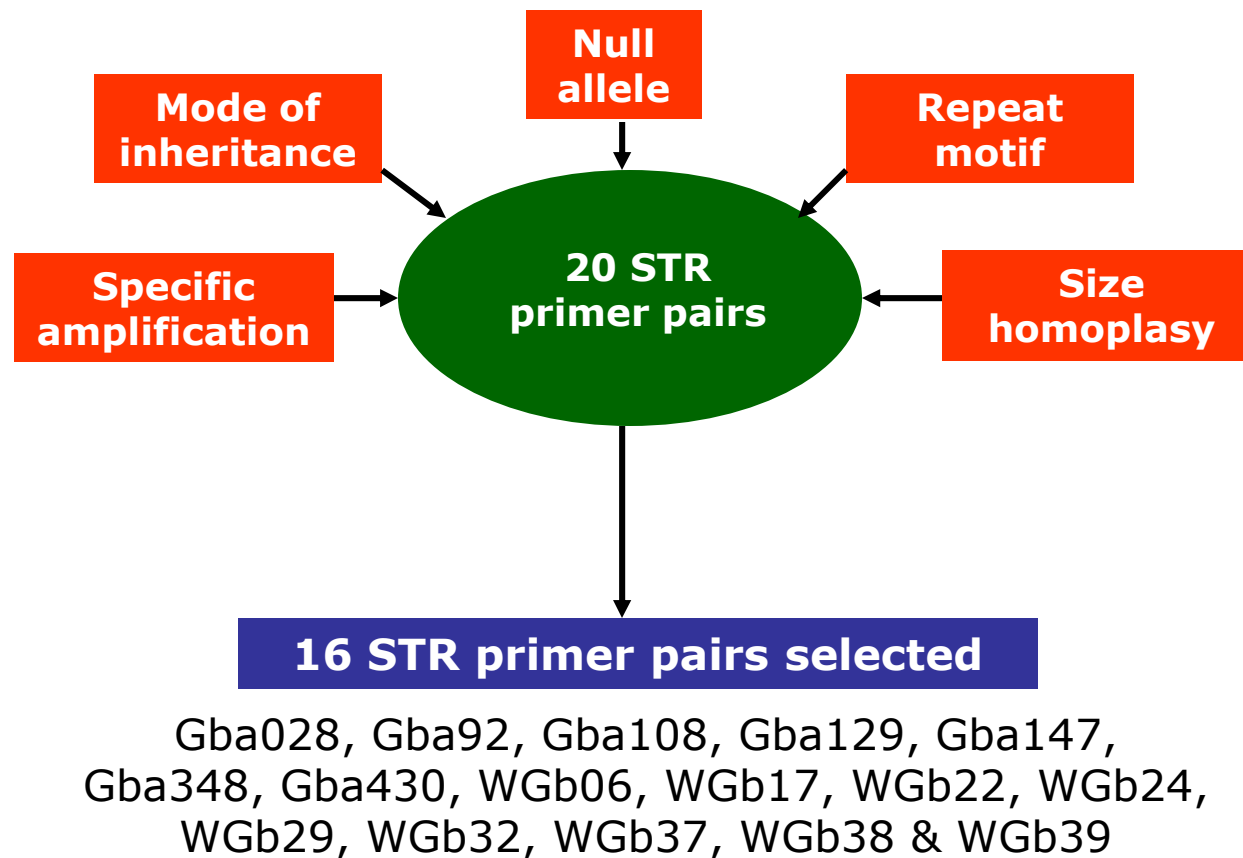
Haplotype	<i>ycf9-trnG</i>					<i>psbM-trnD</i>		<i>trnG-rps14</i>				
	214	235	261-267	283-289	326-327	352-355	98	128	194	146	323-328	358
P1	C	C	ATTCTAA	TTTAGAA	G	C	G	A
P2	A	C	ATTCTAA	TTTAGAA	G	T	G	TTTATT	A
P3	A	C	ATTCTAA	TTTAGAA	G	T	G	A
P4	A	A	ATTCTAA	T	G	T	G	A
P5	C	C	ATTCTAA	TTTAGAA	T	C	G	A
P6	C	C	ATTCTAA	TTTAGAA	G	C	G	A
P7	C	C	ATTCTAA	TTTAGAA	T	G	C	G	A
P8	A	C	ATTCTAA	TTTAGAA	G	T	G	A
P9	C	C	ATTCTAA	TTTAGAA	G	C	G	A
P10	A	C	ATTCTAA	TTTAGAA	G	T	G	A
P11	A	C	ATTCTAA	TTTAGAA	G	T	G	A
P12	A	C	TTTAGAA	AT	ATT	.	G	T	T	G

Haplotype distribution map

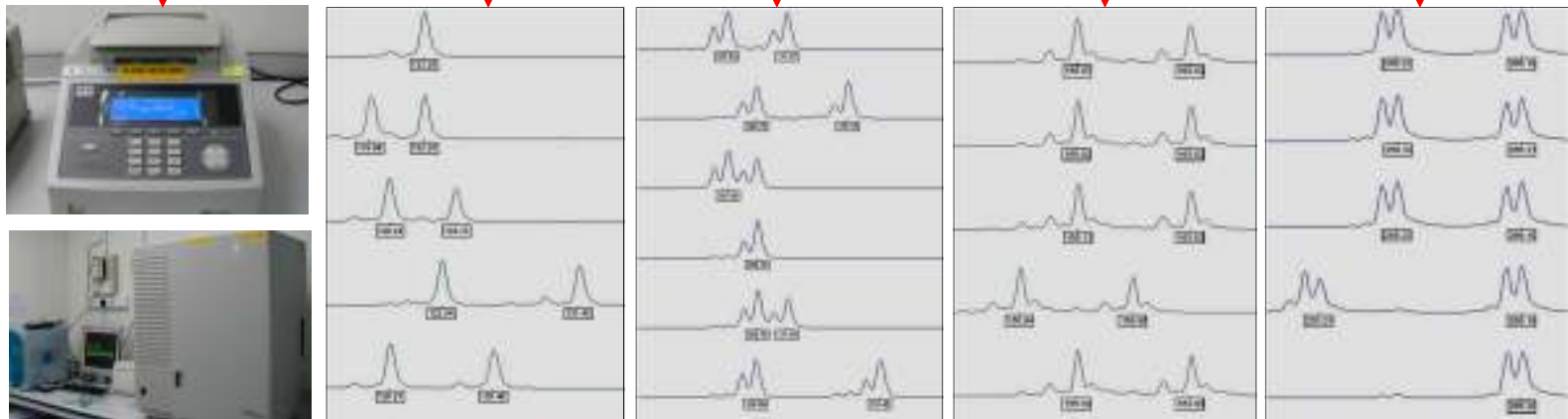


- The map clearly divided Malaysia into West Malaysia (Malay peninsula) and East Malaysia (Sabah and Sarawak)
- Simulation studies (presence/ absence of haplotype) were used to test whether a suspected timber confirms to a given geographical origin
- Overall, the observed type I (false positive) and II (false negative) errors showed good concordance with the predicted 5% threshold

DNA database for individual identification



16 selected STR markers



747 samples collected throughout Malaysia

Probability of exclusion > 0.9999 could be achieved using the 16 STR markers

Characterization of individual identification databases

Database	Coancestry (θ)	Inbreeding (f)
Malaysia	0.121*	0.027*
West Malaysia	0.067*	0.009*
East Malaysia	0.065*	0.035*

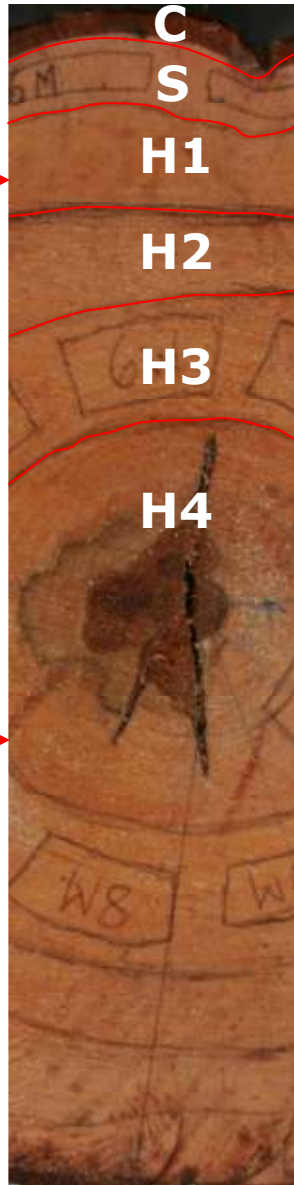
- All the databases showed significant levels of coancestry (θ) and inbreeding (f) coefficients
- Similar to *N. heimii* (Tnah et al. 2010), for the estimation of profile frequency, the subpopulation-cum-inbreeding model by Ayres and Overall (1999) can be adopted for *G. bancanus*

$$\begin{aligned} \text{Homozygote:} \\ P(A_i A_i | A_i A_i) = & \frac{[\theta + (1 - \theta)p_i]}{f + (1-f)[\theta + (1 - \theta)p_i]} f^2 + 2f(1-f) \frac{[2\theta + (1 - \theta)p_i]}{(1 + \theta)} \\ & + (1-f)^2 \frac{[2\theta + (1 - \theta)p_i][3\theta + (1 - \theta)p_i]}{(1 + \theta)(1 + 2\theta)} \end{aligned} \quad 1$$

$$\begin{aligned} \text{Heterozygote:} \\ P(A_i A_j | A_i A_j) = & 2(1-f) \frac{[\theta + (1 - \theta)p_i][\theta + (1 - \theta)p_j]}{(1 + \theta)(1 + 2\theta)} \end{aligned} \quad 2$$

It is possible to extract quality DNA from dry wood?



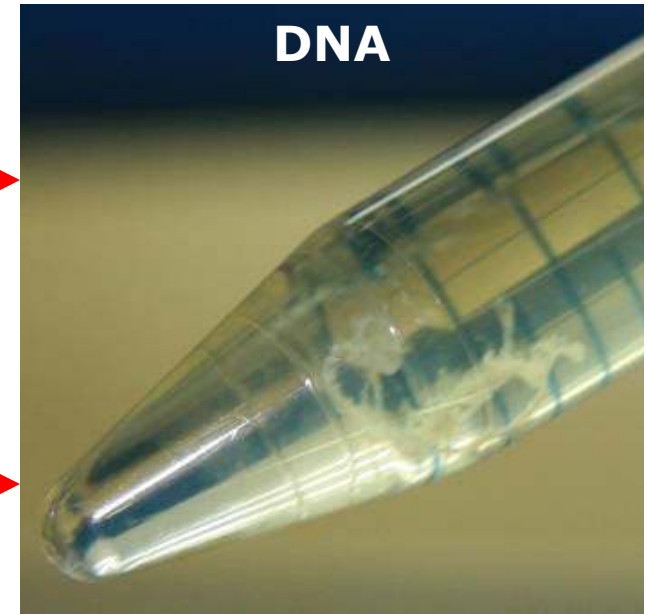


PROTOCOLS

- 1) Qiagen
- 2) CTAB

DURATION

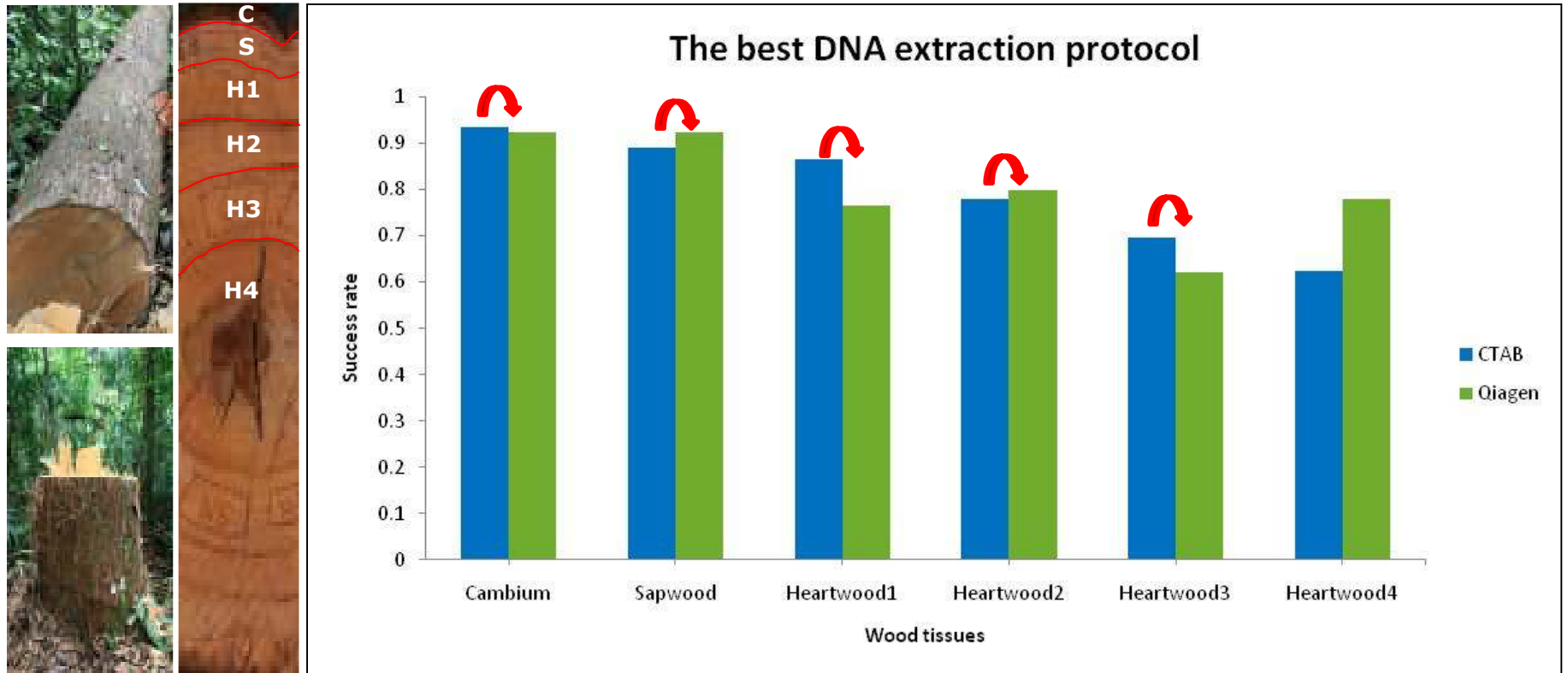
- 1) 2W
- 2) 4W
- 3) 6W
- 4) 8W
- 5) 4M
- 6) 6M
- 7) 8M
- 8) 10M
- 9) 12M



QUALITY QUANTIFIED

- 1) One cpDNA region:
trnE-trnT)
- 2) Four STR loci:
Gba092, Gba100,
Gba175 & WGb17

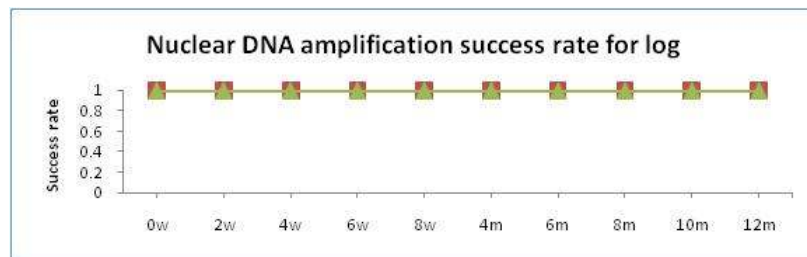
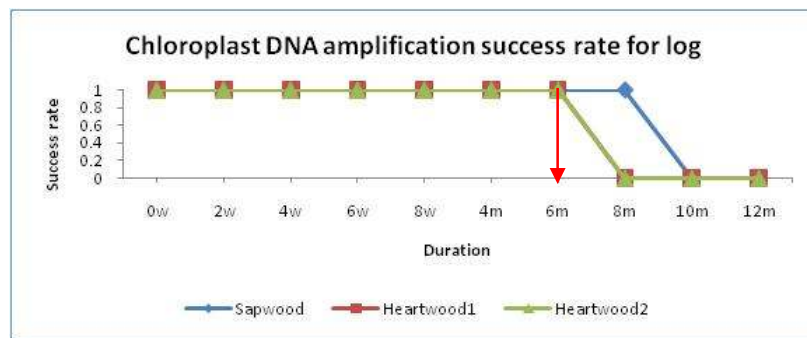
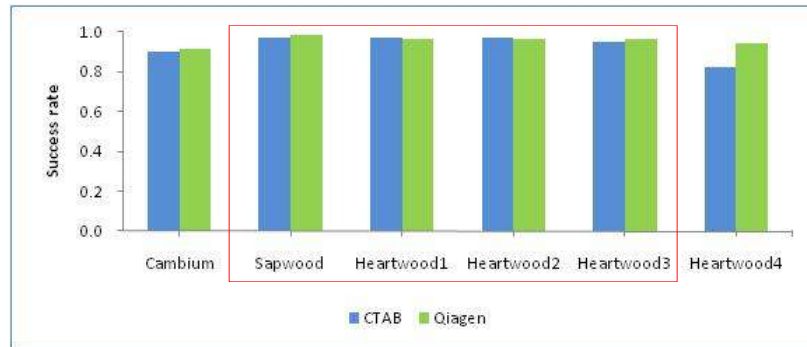
Which protocol is better?



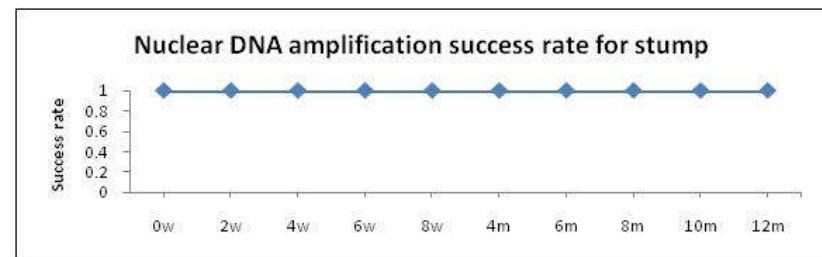
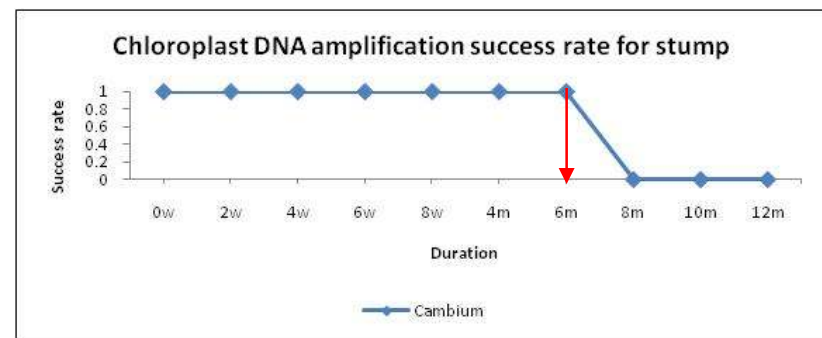
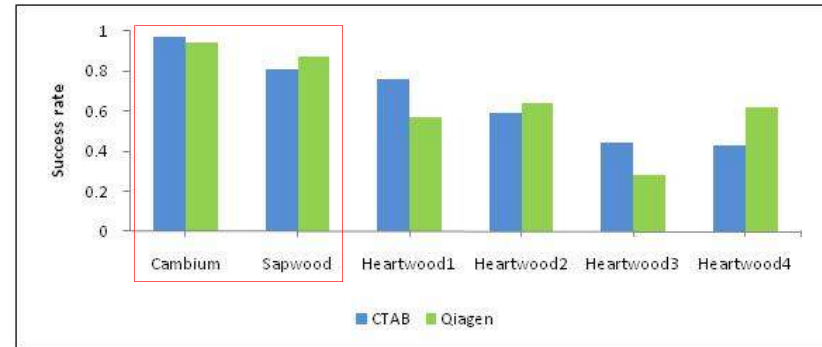
Both the modified CTAB and DNeasy Plant Mini kit (Qiagen) can be used to extract DNA from dry wood of *G. bancanus*

Does preservation of wood affect DNA quality?

Log



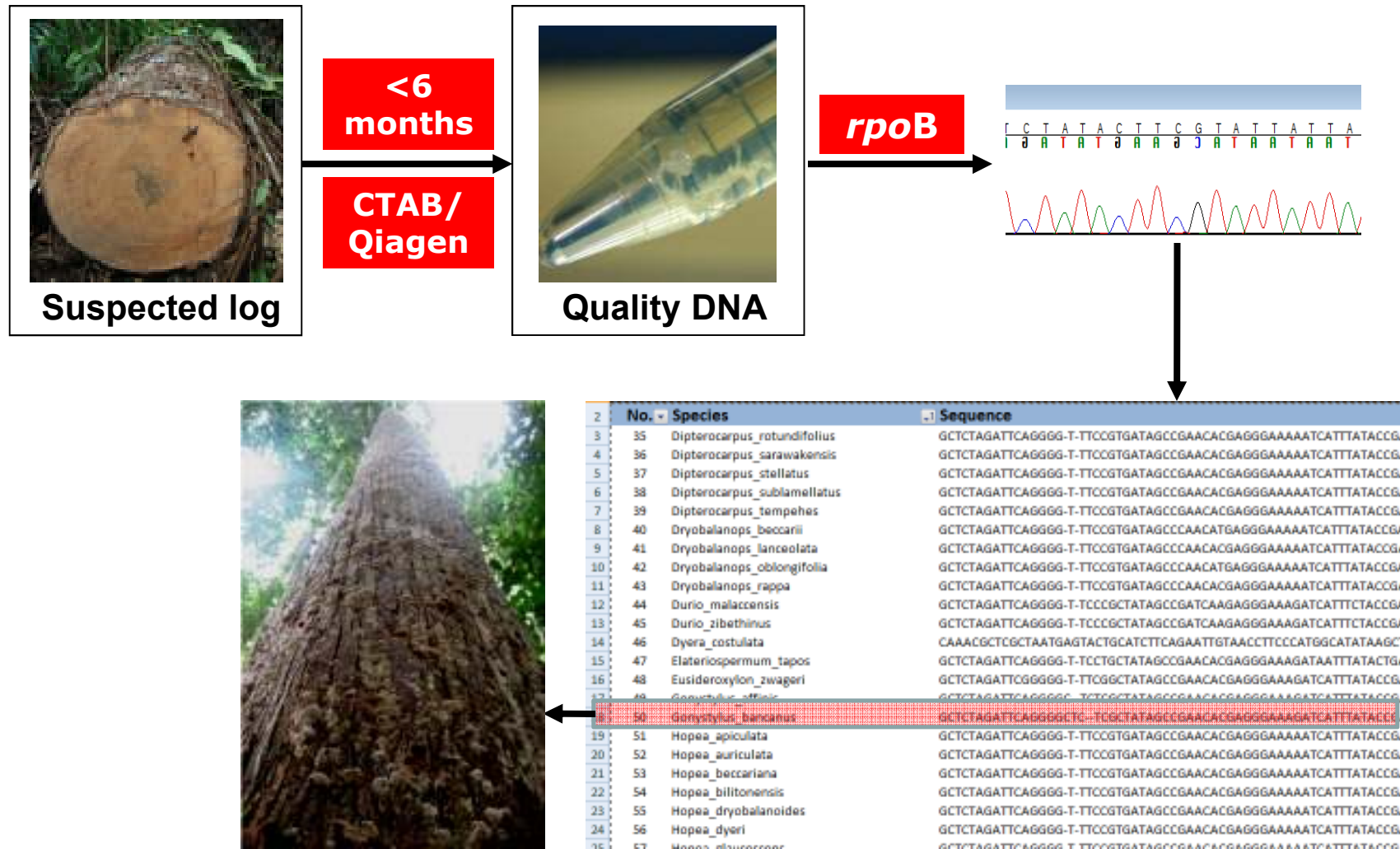
Stump



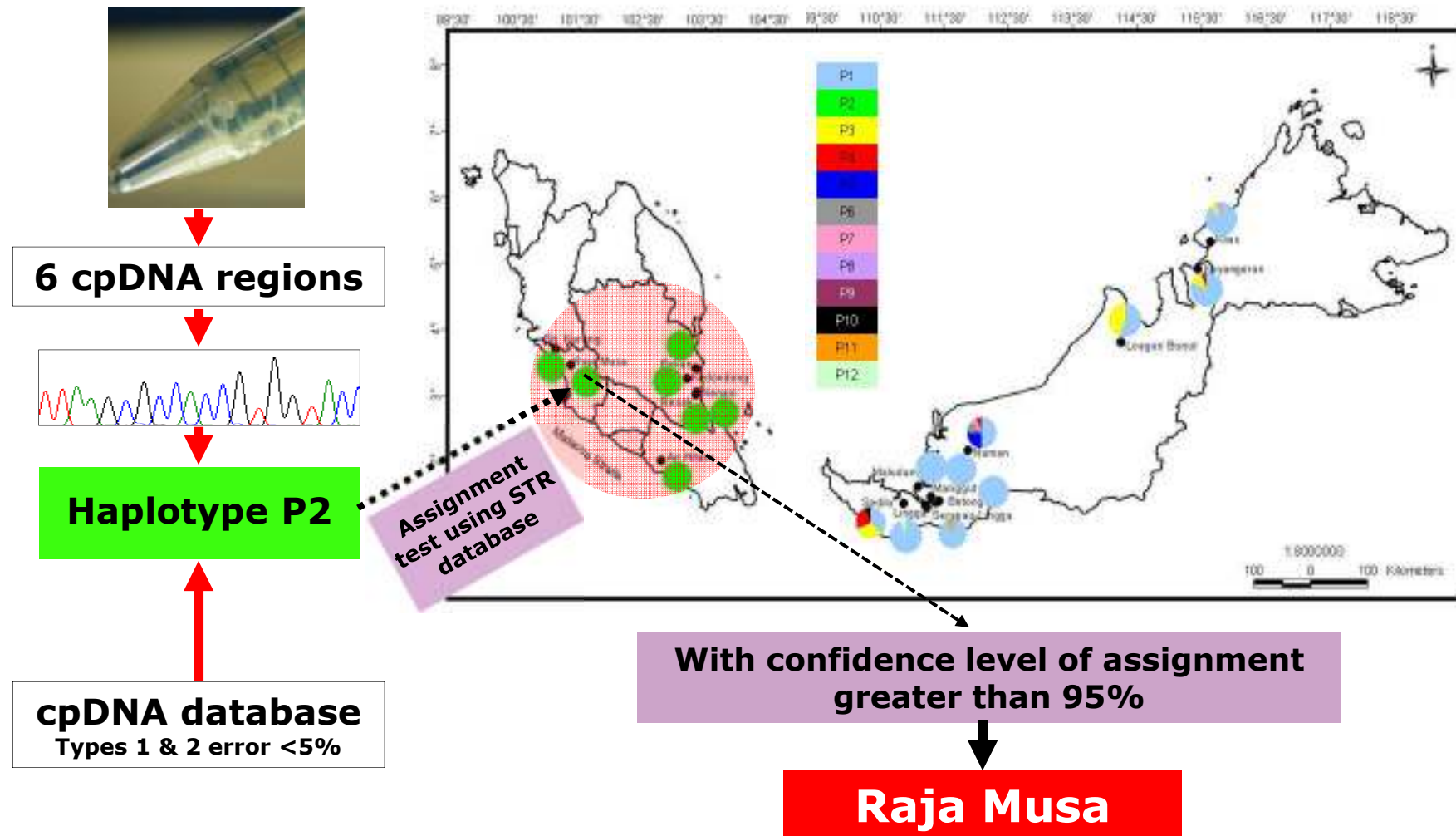
Yes, it is recommended that DNA extraction from the wood should be carried out within 6 months after felling for log and stump

Applications of the databases

To identified the species of suspected log

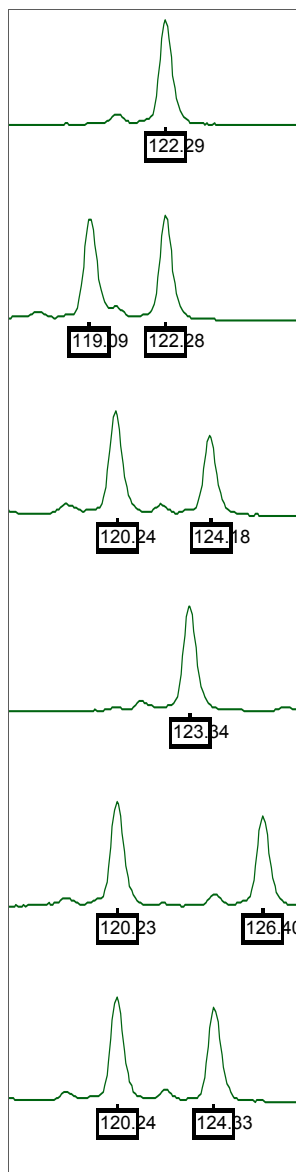




To trace suspected log into its population origin

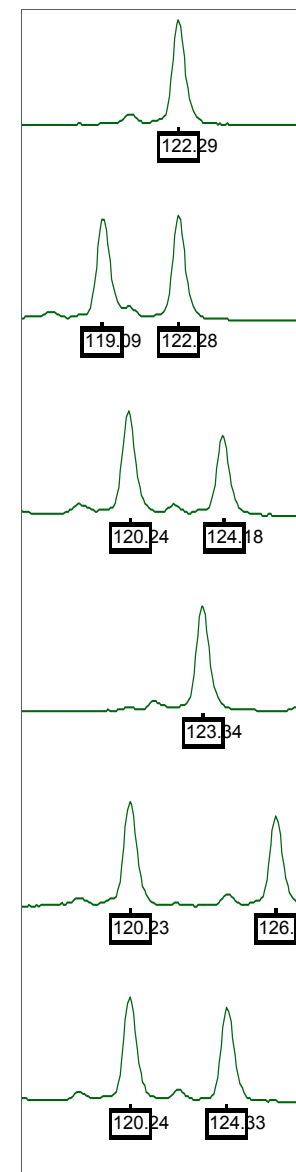


To match the suspected log into its original stump





Locus	 Genotypes	
	 Genotypes	
1) Gba028	246/252	246/252
2) Gba092	088/104	088/104
3) Gba108	182/186	182/186
4) Gba129	157/172	157/172
5) Gba147	140/140	140/140
6) Gba348	114/114	114/114
7) Gba430	124/134	124/134
8) WGb06	199/199	199/199
9) WGb17	339/346	339/346
10) WGb22	280/284	280/284
11) WGb24	341/349	341/349
12) WGb29	135/135	135/135
13) WGb32	293/303	293/303
14) WGb37	162/177	162/177
15) WGb38	275/283	275/283
16) WGb39	156/160	156/160



Using database to extrapolate the possibility of a random match

West Malaysia DNA profiling database (Allele frequencies)

Subpopulation-cum-inbreeding model ($\theta = 0.067$; $f = 0.009$)

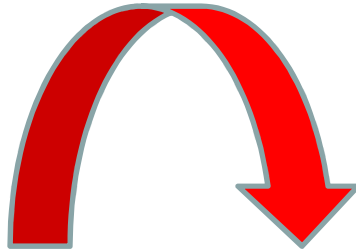
Locus	Genotype		Allele frequencies		Formula	Genotype frequency
	Allele 1	Allele 2	Allele 1 (p)	Allele 2 (q)		
Gba028	246	252	0.211	0.354	2	0.171
Gba092	88	104	0.132	0.348	2	0.122
Gba108	182	186	0.125	0.021	2	0.026
Gba129	157	172	0.463	0.277	2	0.266
Gba147	140	140	0.568	0.568	1	0.402
Gba348	114	114	0.268	0.268	1	0.144
Gba430	124	134	0.018	0.057	2	0.017
WGb06	199	199	0.998	0.998	1	0.997
WGb17	339	346	0.066	0.020	2	0.018
WGb22	280	284	0.107	0.409	2	0.123
WGb24	341	349	0.350	0.361	2	0.260
WGb29	135	135	0.605	0.605	1	0.444
WGb32	293	303	0.163	0.278	2	0.117
WGb37	162	177	0.302	0.082	2	0.082
WGb38	275	283	0.052	0.266	2	0.060
WGb39	156	160	0.104	0.716	2	0.197

Profile frequency

3.991×10^{-15}

Random match probability

1 in 2.505×10^{14}



99.9999999...% sure that the log is originated from this stump

**Provide legal
evidence to convict
the illegal loggers
under the Section
15, National
Forestry Act 1984
(amended 1993)**



**Upon conviction,
liable to a fine not
exceeding RM500k
and to imprisonment
not less than 1 year
but not exceed 20
years**

Summary

By using Chengal as the model, we had demonstrated for the first time on

How to establish DNA database for species identification?

How to establish DNA database for population identification?

How to establish DNA database for individual identification?

How to extract quality DNA from dry wood/wood product?

Subsequently, the same methodologies were successfully been used to establish the timber tracking systems for:



ACKNOWLEDGEMENT

The Forest Departments of Peninsular Malaysia and State Forest Departments of Peninsular Malaysia, Sarawak & Sabah are duly acknowledged for granting us the permission to access the forest reserves as well as logistic assistance

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Thank you for your attention

Fighting illegal logging through science,



for biodiversity conservation and sustainable use of forest resource