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CAMPUS DE ILHA SOLTEIRA

BÁRBARA ROCHA VENANCIO

CHAIN OF CUSTODY CONTROL OF IPE TIMBER (*Handroanthus* sp.) FROM THE AMAZON RAINFOREST, USING DNA FINGERPRINTING

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BÁRBARA ROCHA VENANCIO

CHAIN OF CUSTODY CONTROL OF IPE TIMBER (*Handroanthus* sp.) FROM THE AMAZON RAINFOREST

Dissertation presented to the Engineering Faculty of Ilha Solteira – UNESP as part of the requirements to obtain the title of Master in Agronomy. Specialization: Production Systems.

Prof. Dr. Alexandre Magno Sebbenn
Supervisor

Dr. Bernd Degen
Co-supervisor

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AUTORA: BÁRBARA ROCHA VENANCIO
ORIENTADOR: ALEXANDRE MAGNO SEBBENN
COORIENTADOR: BERND DEGEN

Aprovada como parte das exigências para obtenção do Título de Mestra em AGRONOMIA, especialidade: SISTEMAS DE PRODUÇÃO pela Comissão Examinadora:

Prof. Dr. ALEXANDRE MAGNO SEBBENN
Secretaria do Meio Ambiente do Estado de São Paulo / Instituto Florestal de São Paulo - IFSP

Prof. Dr. MARIO LUIZ TEIXEIRA DE MORAES
Departamento de Fitotecnia, Tecnologia de Alimentos e Sócio Economia / Faculdade de Engenharia de Ilha Solteira - UNESP

Profa. Dra. CELINE BLANC-JOLIVET
Departamento de Genética Florestal / Thunen Institute of Forest Genetics

Ilha Solteira, 20 de abril de 2017

DEDICATION

I dedicate this work to God, to the people who kept me going when I wanted to give up and to you reader.

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“I can’t go on. I’ll go on. ”
Samuel Beckett
The Unnamable.
Grove Press, English edition in 1958.

RESUMO

A presente dissertação de mestrado é composta por uma seção introdutória, seguida de uma revisão da literatura a qual antecede os três capítulos subsequentes. O primeiro capítulo aborda um conjunto de revisões de conhecimentos científicos contemporâneos sobre os efeitos da exploração madeireira em florestas tropicais e as práticas madeireiras utilizadas no Brasil, quais têm se demonstrado insuficientes para garantir a sustentabilidade tanto na produção genética quanto na produção madeireira. O segundo capítulo é um "primer note" descrevendo a identificação de 402 loci putativos (polimorfismos de nucleotídeo único – SNPs, inserções / deleções - INDELs) para Ipe (*Handroanthus* sp.), destinado à estudos de genética de populações, filogeografia e DNA fingerprinting. O último capítulo discute a viabilidade de DNA fingerprinting para espécies do gênero *Handroanthus*. Esse traz a análise da diversidade genética, diferenciação genética de populações de *Handroanthus* sp., bem como entre os países de origem das amostras, análises de auto atribuição de genótipos e testes de atribuição de madeira ao local de origem.

Palavras-chave: Comércio de madeira. Controle de madeira. Desenvolvimento de marcadores. DNA fingerprinting. *Handroanthus* sp. Manejo florestal sustentável. MassARRAY. Polimorfismos de nucleotídeo único – SNP. Seleção disgênica.

ABSTRACT

The present master dissertation is composed by an introductory section, followed by a review of literature, which prefaces the three subsequent chapters. The first chapter of this dissertation is a review assembly contemporary scientific knowledge about the effects of the forest logging in tropical rainforests and the actual logging practices used in Brazil, which seems insufficient to ensure sustainability in both genetic and timber production aspects. The second chapter is a primer note describing the identification of 402 putative loci (single nucleotide polymorphisms –SNPs; and insertion/deletions- INDELs) for Ipe (*Handroanthus* sp.), intended to help population genetics, phylogeography and DNA fingerprinting studies. The last chapter discuss the feasibility of DNA fingerprinting for *Handroanthus* species. It brings genetic diversity analysis, genetic differentiation of *Handroanthus* sp. sample-populations, as well as among countries, self-assignment and timber assignment tests analysis.

Keywords: DNA fingerprinting. Dysgenic selection. *Handroanthus* sp. Marker development. MassARRAY. Single nucleotide polymorphism – SNP. Sustainable forest management. Timber tracking. Timber trade.

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1 INTRODUCTION

The rapid deforestation is a fact in most tropical regions, and many tropical forest biomes have been reduced to forest fragments, or turned into pastures or areas of agricultural crops (ASNER et al., 2005;. SEBBENN et al., 2008 ; LACERDA et al., 2013). Selective logging followed by clear-cutting and fire is the main factor of change in the Amazon rainforest. The impacts of deforestation on forest species are sharp, tree populations at these locations are lost and species populations in adjacent areas are isolated in forest fragments of varying sizes (SEBBENN et al., 2008). Therefore, we can expect a reduction in fitness of these populations to environments and a decrease in adaptive genetic potential (LOWE et al., 2005). The long term viability of the forest industry depends on the forest's capacity to recover in quality and volume of wood (LACERDA et al., 2013), which is partially determined by both genetic diversity of forests and the use of sustainable management methods for the production of wood (LOWE et al., 2005; SEBBENN et al., 2008; LACERDA et al., 2013).

It is estimated that the illegal trade represents a total of 30-100 billion dollars per year (THE ECONOMIST, 2012), and approximately 50% of the timber sold from the Amazon Basin, Central Africa, Southeast Asia and the Russian Federation (GOLÇALVES et al., 2012). There is a great difficulty and high cost of monitoring the chain of custody (CoC) and the declared wood species that are been traded due to documentation counterfeiting and corruption. DNA fingerprinting allied to phylogeography and population genetics are revolutionizing the identification of individual organisms and their origins as result of the impossibility of falsify DNA, which is an intrinsic property of living-beings.

The DNA fingerprinting technique, also known as individualization, permits individuals differentiation. DNA fingerprinting combined to another technique named DNA barcode (enables botanical identification of animals and plants; e.g.: genus and species) allows tracking the source of plants, fungi, and other organisms and also their descendants, but also distinguishing individuals and species. Due to the high potential of these techniques, they have been studied as forensic tools to detect the geographic origin and to monitor the custody chain of timber species (TNAH et al., 2009; LOWE; CROSS, 2011; JOLIVET; DEGEN, 2012; TNAH et al., 2012; DEGEN et al., 2013; NG et al., 2016). This technic

combination can be employed for controlling of illegally sourced timber trade, as well as those species listed as being at risk of extinction and prohibited of exploitation.

Among the many tree species exploited for timber production, species of the genus *Handroanthus*, are an example of species logged in Latin America (occurring from Argentine to Mexico). The timber commonly named as "Ipe", Pau D'arco or Lapacho, includes different species of the genus *Handroanthus*. The great interest in Ipe timber is due to its exceptional physical properties and high commercial value, being called "new mahogany" (SCHULZE et al., 2008). The preservation of the structure; demography and genetic diversity in logged populations are crucial to protect forest ecosystems and sustainable timber production. The use of reliable species and custody chain control techniques, such as DNA barcode and DNA fingerprinting, is the key to effectively curb issues such as trade in timber and timber products from illegal sources, and therefore the rapid deforestation and deterioration of the Amazon and other natural forests.

Since 2014 a 39 months project named "Large Scale Project On Genetic Timber Verification" (LSP) funded by the German Federal Ministry of Food and Agriculture has been carried on in South America and Africa. This initiative aims the creation of a genetic reference data to check the country of origin of 14 valuable timber species from South America and Africa. The database will be implemented in the Global Timber Tracking Network (GTTN). Ipê timber is among the studied timber species of the LSP. In the frame of this project the development of a timber control system based on DNA fingerprint of the *Handroanthus* genus should provide regulatory bodies, operators and timber traders an effective tool to achieve control of the chain of custody and also provide evidence to prove the origin declared.

2 REVIEW OF LITERATURE

2.1 *HANDROANTHUS* GENUS

Species of the *Handroanthus* Mattos genus belongs to the botanical family Bignoniacaea. Bignoniaceae family comprises about 82 genera and 827 species, distributed throughout various biomes, present in the Amazon, Savanna, Atlantic Forest. *Handroanthus* is one of the most commonly found genera of Bignoniaceae in Neotropical regions. *Handroanthus*, formally part of *Tabebuia* genus, has a complex and long taxonomic history due to its anatomical and morphological variety. In 2007, Grose and Olmstead (2007a) reclassified part of the *Tabebuia* species under the *Handroanthus* genus, as a result of molecular and phylogenetic studies.

Handroanthus species have been widely studied for their medicinal properties, such as antimicrobial, anti-inflammator, anticancerous and antileukemic actions (MOON et al., 2010; TEIXEIRA et al., 2014), and most of the available genetic information is linked to this fact. Even though the number of sequences available in public database was still negligible.

The great interest in *Handroanthus* (Ipe) timber is due to its wood that is among the heaviest and hardest known in the world, leading to a high commercial value in the international market (GROSE; OLMSTEAD, 2007b). The cubic meter of Ipe timber reached $ 1,305 USD in February 2015 (lumber for export), the most exported Brazilian timber in volume and second highest commercial value, surpassing Jatobá (*Hymenaea* spp.), Muiracatiara (*Astronium lecointei*) and Maçaranduba (*Manilkara* spp.) (ITTO, 2015). A substantial amount of Ipe timber from South America is exported to the European Union (EU) and United States of America (USA). In Germany, tropical decks typically of Bangkirai have been replaced by Latin American species of higher quality, as Garapa, Cumaru and Ipe (ITTO, 2012). In 2014, Ipe timber volume imported by the US was of 29.328 m^3, being the third most imported tropical timber species and the most valuable compared to other exported Brazilian timbers (ITTO, 2015). However, generally *Handroanthus* species are not distinguished commercially, according to Schulze et al. (2008) most of the Ipe timber harvested in Brazil is represented by *H. impetiginosus* ((Martius ex A. P. de Candolle) J.R. Mattos) and *H. serratifolius* ((Vahl) S. Grose). The same study, that evaluated the impacts of logging in the Brazilian Amazon, concluded that *H. impetiginosus* and *H. serratifolius* are

threatened due to the high intensity of exploitation, even if the reduced-impact logging (RIL) harvesting practices. For this reason, these species need additional protection under the forest legislation.

2.2 TREATIES AND REGULATIONS CONCERNING TIMBER TRADE

One of the most notable international agreements is the Convention on International Trade in Endangered Species of Wild Fauna and Flora, widely known as CITES, with 182 signatory countries (CITES, 2016). Brazil joined CITES in 1975, through Decree No. 76.623, and its provisions were implemented in 2000, through Decree 3.607. Decree 3.607 of September 21, 2000 assigned IBAMA as administrative in charge (responsible for international trade licenses) and as Scientific Authorities the Rio de Janeiro Botanical Garden, ICMBIO and also IBAMA (BRAZIL, 2000), among other measures.

The European Union aiming to eliminate illegally sourced wood and wood products from its market, and to encourage producers and importing countries to implement control policies in the custody chain, developed and put into practice the European Timber Regulations (EUTR), which came into force in March 2013. Among other requirements, the EUTR requests the presentation of information and full documentation on the product in question; the documents must be in accordance with current legislation in the country of origin of the timber product (EUROPEAN UNION, 2010). Similar requirements to the EUTR are in place in the USA, through the Lacey Act, in force since May 22, 2008 (UNITED STATES OF AMERICA, 2008). This regulation comprises actions to ban in the USA or abroad the trade of plants and their products illegally sourced. The Lacey Act also establishes penalties for violations of the law, including the confiscation of property, fines and imprisonment (UNITED STATES OF AMERICA, 2008). As a result of these regulations put into force, logging companies operating in South America are under pressure to provide information, ensuring the correct botanical species and the legal origin of the timber.

Other regulations as "Canadian Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (1992)" and "Australian Illegal Logging Prohibition Act (2012)" are in place in Canada and Australia, respectively, which show that timber consumer countries (among other wild/natural products) have been trying to curb the

import of wood and wood products that have not been obtained in accordance the laws of the origin country.

Illegal logging has a wide range of activities related to the misuse of timber resources. In most countries, specific forms of illegal logging include timber theft of public and private forests or protected areas, harvesting operations outside designated or of protected species, or even tax evasion on the wood and its products. Other activities that may constitute illegal logging in some jurisdictions are stolen timber smuggling, counterfeit documents related to timber harvesting and transport, violation of forest regulations among several other civil and criminal offences (DEGEN; FLADUNG, 2008). Illegal logging is believed to cost the global total trade estimated at $ 15-20 billion USD (GONCALVES et al., 2012). According to the World Bank (2014), it is estimated that 23-30% of the world supply of wood is illegal and this trade reduces wood products prices from 7 to 16%, thus causing disadvantages to products of legal origin (WORLD BANK, 2014).

2.3 ILLEGAL TIMBER CONTROL

Several areas have developed scientific studies aimed at developing reliable methods capable of resolving issues such as the identification of wood species, origin (country or forest concession, etc.) and individual identification for the chain of custody control, one of the main requirements of the regulations mentioned previously. These methods can be classified into three groups: morphological, chemical and genetic methods (DEGEN; FLADUNG, 2008; LOWE, 2008; ZAHNEN, 2008; DORMONTT et al., 2015).

Among the more established methods are dendrology, dendrochronology and wood anatomy, both visual methods. The dendrochronology science is based on the study of the growth rings of trees, which are distinguished mainly in temperate species, commonly used to estimate the age of the tree and clarify past climate changes; they contain information about the environmental conditions at the time of its growth. It is a visual method with potential for use in forensic wood analysis, because it allows estimating the date of falling of the tree (whether the part contains the most outer rings and bark) and the distinction between individuals when patterns of rings are compared (DORMONTT et al., 2015). This technique can be limited to use with tropical species, as distinct growth rings are rare (DORMONTT et al., 2015).

The science of analysis of wood structures, known as wood anatomy, has traditionally been used to elucidate the taxonomy issues and is widely accepted as a forensic tool. This methodology allows the rapid identification of the genus, being widely used in the field, but may be limited in certain cases regarding the identification of the samples at the species level (DORMONTT et al., 2015). The anatomical characteristics can be influenced by several factors, such as genetic and environmental factors. International Association of Wood Anatomists (IAWA) presents the standards to be followed and have the division called "Wood Research Against Illegal Timber Harvest" (WRAITH). WRAITH handles issues related to illegal logging and forensic wood science (JANSEN; SCHOLS; STEVEN, 2016). There is also timber automated anatomical analysis that prospects for increased accuracy and easy identification at the species level, but for this, the development of a representative data base of the natural variation of species is required (DORMONTT et al., 2015).

Chemical methods include the mass spectroscopy of stables isotopes, of phytochemical and near infrared spectroscopy (NIR) and stable isotopes technique. Mass spectrometry is combined with the statistical analysis of the chemical profile of phytochemicals for obtaining evaluation set forth for heartwood. This phytochemical evaluation allows the identification of various taxonomic levels, as demonstrated by Cabral et al. (2012) in *Swietenia macrophylla* distinction (CITES species) of six other visually similar wood species, but taxonomically distant. In the compilation developed by Dormontt et al. (2015), other studies are cited; these studies support the possibility of using this method for species differentiation and the potential to estimate the provenance in some cases.

The NIR, such as mass spectrometry, analyses the phytochemical properties, being widely used for physical-mechanical characterization of wood. This technique aims at characterizing the timber through absorption of electromagnetic rays when exposed to near infrared. Due to its simplicity of application this technology has been studied for use in the taxonomic species identification (DORMONTT et al., 2015).

During the synthesis of phytochemicals compounds, stable isotopes are incorporated into the plant. These stable isotopes have a specific ratio relative to its availability in the surrounding environment, thus allowing the attainment of information concerning the place of origin or even determining the geographical origin of the wood sample (DORMONTT et al., 2015). Stable isotopes associated with other elements create a unique isotopic pattern of a given area (DORMONTT et al., 2015). The use of stable isotope technique has been gaining

strength and visibility as a forensic tool in identifying the timber origin as a result of its high accuracy.

The third category of new techniques aiming forensic diagnosis of species and wood origin is the genetic methods. DNA Barcoding is used for determining species, genetic assignment to determine the geographical origin, and finally the DNA fingerprinting to differentiate individuals.

DNA barcoding and multiloci approaches based on nuclear and chloroplast microsatellite, as well as SNP (Single Nucleotide Polymorphism) markers are the main methods used to determine species or distinguish among different species (DEGEN; FLADUNG, 2008). This method was first applied in animals and can be applied also for the identification of tree species (DEGEN; FLADUNG, 2008). A large study successfully carried out developed a reference library of tropical forests in India using the Standard barcode labels (in plants are the rbcL, matK and trnH-psbA - plastid markers and the nuclear marker ITS) (DEGEN; SEBBENN, 2014; NITHANIYAL et al., 2014; SMITHSONIAN - NATIONAL MUSEUM OF NATURAL HISTORY, 2014). There are several databases for DNA barcoding as the Barcode of Life Database - BOLD, which aims to build a public reference library of species identifiers that could be used to designate the unknown samples from known species (BARCODE OF LIFE, 2016).

Genetic assignment (based on population genetics and phylogeography) and DNA Fingerprint will be described on the following topics.

2.3.1 Genetic assignment

Approaches based on population genetics and phylogeography have been used to determine the geographical origin of individual trees. This is achievable because natural forests usually present a genetic structure in local and regional scales (DEGEN; SEBBENN, 2014), called spatial genetic structure (SGS). SGS is associated with the non-random distribution of alleles and genotypes in space; usually individuals of a species that are located close tend to be more genetically related to each other in comparison with more distant individuals (DEGEN; SEBBENN, 2014; DORMONTT et al., 2015). This particular structure is mainly influenced by the mating system, pollen and seed dispersal patterns as well as vegetative propagation (CAVERS et al., 2005; DEGEN; SEBBENN, 2014). However large

SGS is directed influenced geographical, landscape and climate changes through the history. Events such barrier effect of Amazonian rivers; the isolation of humid rainforest blocks separated by dry forests, savannahs and other intermediate vegetation; changes in canopy-density due to climatic reversals among many other theories might explain genetic differentiation, isolation and even speciation (HAFFER, 2008). Degen and Sebbenn (2014) report that tree species that have the wind as seed dispersal vector and have low population density tend to have larger SGS distance compared to species with other seed dispersal and population density. The use of genetic markers to detect the geographical origin of samples depends on allele's frequencies and genetic differentiation within and among populations in the distribution area of the species. Furthermore it's crucial the quality of genetic reference database (genetic distribution, quantity, used markers and possibly species included) and on the geographic distance between the geographical origin of the unknown timber sample and the next sampled population in the reference database (DEGEN; FLADUNG, 2008). Screening individuals of many populations of a given species with appropriate genetic markers, genetic maps can be developed and used to assign the origin of unknown individuals (DUTECH et al., 2003; DEGUILLOUX et al., 2004; LOWE et al., 2004), as well as to assign individuals or woods to their correct species group (DUMINIL et al., 2006).

Several studies have confirmed the potential of this approach to determine the origin of timber, as the study by Degen et al. (2013) for *S. macrophylla*, Tnah et al. (2012, 2009) for *Neobalanocarpus heimii*, and Jolivet and Degen (2012) that were able to determine with precision of dozens of kilometres the origin for the species *Entandrophragma cylindricum.*

2.3.2 DNA fingerprinting

The technique known as DNA fingerprinting or DNA profiling allows the recognition of individuals or "individuation", and it is the main genetic method applied with forensic purposes with humans (JOBLING; GILL, 2004). To develop a DNA fingerprint several genetic markers (minisatellites, microsatellites, SNPs, etc) must be identified to produce a unique pattern to an individual. To employ this technique to support the illegal timber control, a representative reference sample database is required, allowing a reliable estimation of the probability to obtain an identical profile from a non-related individual of the given tree, that is usually extremely low (DORMONTT et al., 2015). Microsatellite loci are widely used in

humans, however with relatively limited application for wood due to DNA high degradation level. A successful option to degraded DNA material is the use of SNPs. DNA fingerprinting may be cumbersome in plants due to the polyploidy, making interpretation of results more complex (DORMONTT et al., 2015).

Despite several illegal timber control policy, these are not "law-violation proof". One of the requirements of national and international regulations for timber trade is the chain of custody control (CoC). The CoC is mainly based on paper identification for harvested trees in order to trace them along the supply chain, from the forest concession to timber trader (DEGEN; FLADUNG, 2008; LOWE et al., 2010). The weakness of this method is the possibility of forgery, particularly between forest concessions and sawmills, where most of the illegally sourced timber is introduced into the supply chain (LOWE et al., 2010; HÖLTKEN et al., 2012). The DNA fingerprinting is an inherent property of all living being, including trees, and cannot be falsified (DEGEN et al., 2013).

This technology can be used to verify the integrity of the chain of custody declared for suspected of being illegally sourced, as demonstrated in the study of Lowe et al. (2010). This is possible by collecting samples along the chain of custody, to detect if there was and at what point was the insertion of illegal timber into the lots. This technique has already been used as certification in Southeast Asia, known as CERTISOURCE (http://www.certisource.co.uk/).

Genetic certification of species and custody chain based on DNA fingerprint and population genetics are among of the most promising techniques to curb illegal timber trade. The biggest advantage of this technique is due to the fact that the DNA cannot be changed and that any product of a given tree will always have the same genetic information as the original individual (THE ECONOMIST, 2012); this property allows the monitoring along the custody chain, frightening corruption.

2.3.3 DNA extraction from timber

Despite the existence of many DNA extraction protocols for fresh material such as leaves, cambium, fruits, etc., or the ready-made DNA extraction kits, DNA extraction from timber has been a challenge. The biggest problems faced are the low purity and quantity of DNA from wood mainly due to extremely degraded DNA in comparison with fresh material (DEGEN; FLADUNG, 2008; LOWE, 2008). This occurs because of the presence of

inhibitors, such as carbohydrates (cellulose), phenols (lignin), increased iron level, among others wood extractives that can inhibit the PCR (polymerase chain reaction) (LOWE, 2008).

Jiao et al. (2015) have succeeded in extracting DNA from timber up to the age of 80 years, however with length reduction of the DNA barcoding regions according to the increase of the sample age. Studies of population genetics, DNA fingerprinting and phylogeography also showed a decrease in the success of amplification when used DNA extracted from wood (DORMONTT et al., 2015). Some studies report the use of short fragments sequencing as being an effective method for analysing degraded DNA (DEGUILLOUX et al., 2004; OGDEN et al., 2008). The use of SNPs it is also proposed as a way to circumvent the problem of degraded DNA (OGDEN et al., 2008; HOLTKEN et al., 2012). Höltken et al. (2012) demonstrated the process to develop molecular markers with identification purposes for great commercial important timber species such as *Swietenia* sp. (listed on CITES appendix II), *Khaya*, *Entandrophragma*, and *Carapa*, presenting a low cost alternative. Lowe et al. (2015) recently described a very promising DNA extraction method for lignified tissues, based on the CTAB method and purifying steps with a ready-made kit, that is an outstanding protocol for forensic focused studies.

2.3.4 SNP markers

Single nucleotide polymorphisms (SNPs) are variants of the DNA sequence occurring when a single nucleotide at a homologous locus differs between members of a species, genus or family (DEGEN; FLADUNG, 2008). This technique has several applications, including gender identity, genetic maps, genetic analysis of the population structure and also DNA fingerprinting. The use of SNPs for forensic studies of illegal timber control is becoming more common. There are different techniques for the development and selection of SNPs genetic markers, as described by Ogden et al. (2008) and more recently by Schroeder et al. (2016) and Pakull et al. (2016).

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3 CHAPTER 1: UNSUSTAINABLE FOREST LOGGING IN THE BRAZILIAN AMAZON

ABSTRACT

The aim of this review is elicit the contemporary scientific knowledge about the effects of the forest logging and illegal logging activities in tropical rainforests. The actual logging practices used in Brazil are insufficient to ensure sustainability in both genetic and timber production aspects, and the scenario is worse in case of illegal logging. The harvesting intensity is very high, removing large individuals from forest populations, along with their genes. Current practices reduce population density, altering its ecology and genetic structure of the remaining reproductive population, resulting in the loss of adaptive traits, low growth vigour and poor stem forms. This affects the ecology, demography, mating system, gene flow and genetics on tropical tree species populations. This review highlight the importance of changes of the Brazilian logging regulations, based on solid scientific studies, to avoid long-term losses in genetic diversity. This paper also points out recent developments in chemical and genetic based methods to distinguish tree species and their origin, which can provide national and international authorities with an efficient and trustworthy tool to impede the illegal timber trade and promote trade with legally sourced timber and timber products, which would also contribute to forest conservation.

Keywords: Dysgenic selection. Forest logging. Genetic diversity. Sustainable forest management. Tropical trees.

3.1 INTRODUCTION

Rapid deforestation is a fact in most tropical regions, including Brazil. Many tropical forest biomes have been reduced to forest fragments or turned into pastures and agricultural crops (ASNER et al., 2005; SEBBENN et al., 2008; LACERDA, NIMMO; SEBBENN, 2013). Logging followed by clear cutting and burning (a common practice in illegal logging activities) is the most common land change in the Brazilian Amazon forest. The impacts of forest clearing are evident; tree populations at these sites are lost and populations of species in the adjacent areas are isolated into forest fragments of varying sizes (SEBBENN et al., 2008). Thus, we can expect a reduction in the fitness of a population for these environments and a decrease in the range of adaptive genetic potential (LOWE et al., 2005). Furthermore, the actual logging rules used in Brazil are insufficient to ensure sustainability in both genetic and timber production aspects. Current practices reduce population density, altering its ecology and genetic structure of the remaining reproductive population, resulting in the loss of adaptive traits, low growth vigour and poor stem forms.

3.2 GENETIC IMPACT OF LOGGING OPERATIONS

The long-term viability of the forest industry in the Brazilian Amazon depends on the capacity of the forest to recover timber volume and quality (LACERDA, NIMMO; SEBBENN, 2013), which is partly determined by both the genetic diversity of the species logged and the use of real sustainable harvesting methods for timber production (LOWE et al., 2005; SEBBENN et al., 2008; LACERDA, NIMMO; SEBBENN, 2013). To face biotic challenges and environmental changes, in terms population adaptability, evolutionary potential and productivity, forests depend on the levels of genetic diversity of a species' population (LOWE et al., 2005). Tree populations with reduced genetic diversity are more susceptible to environmental changes or disease-related losses in productivity, fitness and health (LOWE et al., 2005). However, such factors have received little attention in the Amazon forest management plans that use selective logging as the primary timber production system. Tropical trees are particularly vulnerable to the effects of habitat degradation due to their demographic and reproductive characteristics, generally occurring under low population densities (<1 tree/ha)(SCHULZE et al., 2008a), complex and self-incompatible breeding

systems, high outcrossing rates, and critical connections with pollinators and seed dispersal vectors (LOWE et al., 2005).

The extent of the impacts caused by logging operations is related to the intensity at which a species is harvested, and the most valuable and intensely exploited tree species are likely to be the most affected (LACERDA; NIMMO; SEBBENN, 2013). Legal selective logging and illegal logging remove large individuals from a population (SEBBENN et al., 2008; VINSON et al., 2015a), that likely contribute more to reproduction than smaller individuals, hence reducing the population density and increasing the distance between reproductive co-specific trees (SEBBENN et al., 2008; LACERDA, NIMMO; SEBBENN, 2013; VINSON et al., 2015a) . These changes may alter the genetic structure of the remaining reproductive population due to genetic bottlenecks (decreases in the population size), resulting in a loss of alleles and genetic drift, and consequently affecting the descendent populations. Decreases in genetic diversity and increases in self-fertilization, as a result of reductions in population density, also affect abundance, diversity and behaviour of pollinators (MURAWSKI; GUNATILLEKE; BAWAS, 1994; OBAYASHI et al., 2002; LOWE et al., 2005; LACERDA; KANASHIRO; SEBBENN, 2008a, b; SEBBENN et al., 2008; CARNEIRO et al., 2011; VINSON et al., 2015a). For example, Lowe et al. (2005) reviewed the genetic changes of several neotropical tree species after habitat degradation due to logging and forest fragmentation, reporting increases in progeny inbreeding (75%), differences in reproductive output (70%), and decreases in fitness (100%). As show at the Table 1, decreases in population size, losses on the total number of alleles and heterozygosity were observed in adults and seeds of *B. guianensis*, *Carapa guianensis* and *Hymenaea courbaril*. *C. guianensis*, *H. courbaril* and *J. copaia* presented an increase in the fixation index among seeds and offspring that supports the theory of reduction in outcrossing rates.

Table 1- Genetic diversity and inbreeding of adults and seeds of five tree species pre-and post-logging (Pre/Post).

	n		k		H_o		F	
Species	Pre/Post	Diff	Pre/Post	Diff	Pre/Post	Diff	Pre/Post	Diff
Adults								
B. guianensis[1]	71/28	-61%	36/33	-13%	0.66/0.71	8%	-0.05/-0.14	-180%
C. guianensis[2]	199/49	-75%	10.3/8.3	-19%	0.69/0.65	-6%	0.03/0.06	100%
D. odorata[3]	77/50	-35%	115/104	-10%	-	-	-	-
H. courbaril[4]	130/51	-61%	150/113	-25%	0.61/0.58	-5%	0.14/0.17	19%
J. copaia[3]	256/214	-16%	98/96	-2%	0.82/0.82	0%	0.03/0.03	0%
Seeds/Offspring								
B. guianensis	-	-	-	-	-	-	-	-
C. guianensis[2]	390/391	0.26%	10/10.6	6%	0.68/0.67	-1%	0.03/0.05	66.67%
D. odorata[3]	332/252	-24%	106/106	0%	-	-	-	-
H. courbaril[5]	367/250	-32%	159/84	-47%	0.56/0.54	-4%	0.07/0.25	257%
J. copaia[3]	288/658	128%	96/105	9%	0.83/0.83	0%	0.01/0.02	100%

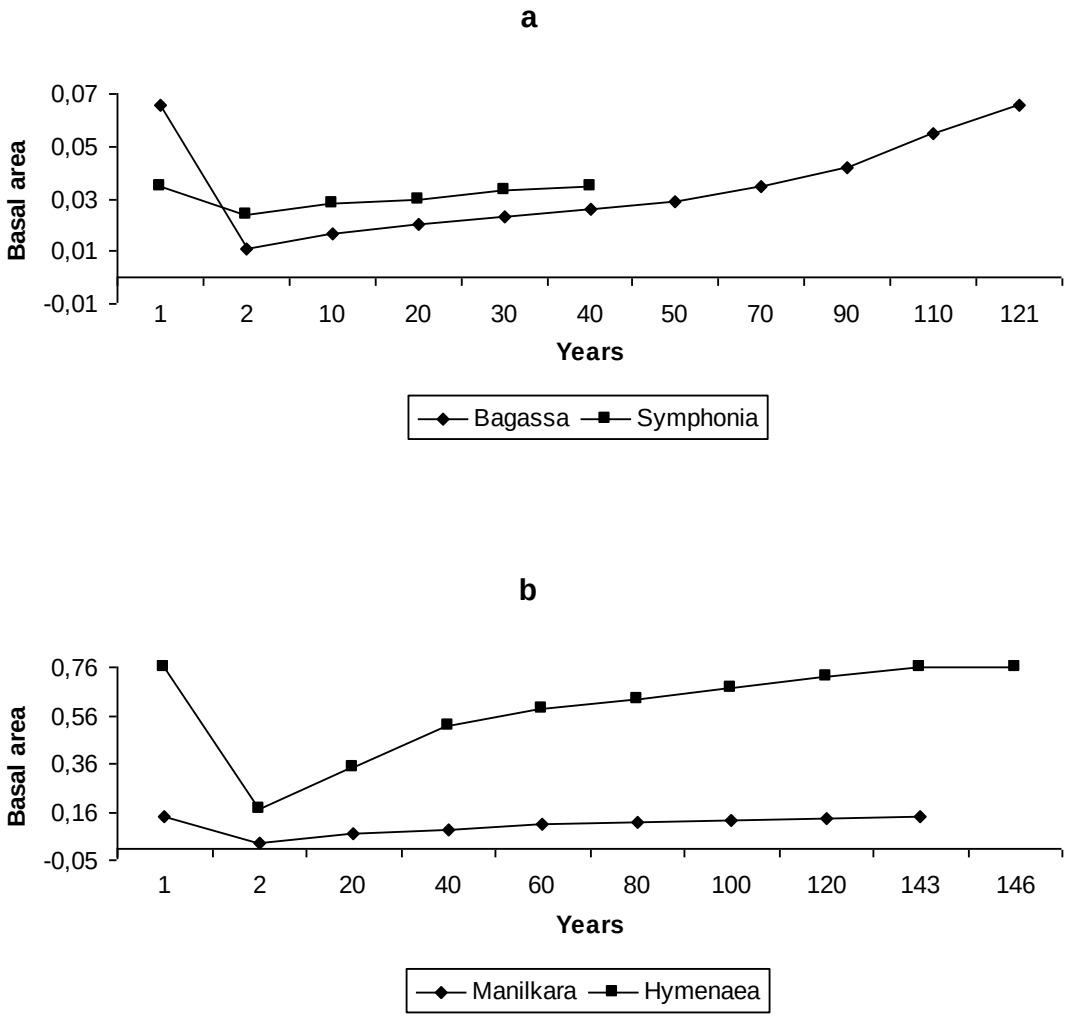
Diff is the difference relative; n is the sample size; k is the total number of alleles; H_o is the observed heterozygosity; F is the fixation index. Sources: [1] -Silva et al., 2008; [2] -Cloutier et al.,2007; [3]- Vinson et al., 2009 ; [4] -Lacerda et al., 2008a. [5]-Carneiro et al., 2011.

3.3 FOREST REGENERATION

Legal forest logging in Brazil is mainly based on the reduced-impact logging (RIL) system, a harvesting approach that is expected to result in a substantial decrease of environmental impacts of tree felling, yarding, and hauling, being a pre-requirement for sustainable timber production (PUTZ et al., 2008; SEBBENN et al., 2008; LACERDA; NIMMO; SEBBENN, 2013). Sustainable forest logging consists of continuous and stable timber production through successive cutting cycles (LACERDA; NIMMO; SEBBENN, 2013). This can be achieved by combining a logging intensity (LI), above a minimum cutting diameter (MCD), with appropriate cutting cycle (CC) length and silvicultural treatments to increase growth rates , thus allowing timber volume to recover to pre-logging levels between

harvesting events (PEÑA-CLAROS et al., 2008; SEBBENN et al., 2008; LACERDA; NIMMO; SEBBENN, 2013). Nevertheless, current Brazilian regulations assume that sustainable timber production can be achieved for all tree species employing a 40-year CC, with a LI of 90% of trees above 50 cm MCD (LACERDA; NIMMO; SEBBENN, 2013). Although the impacts of RIL are less intense compared to traditional non-planned systems, studies have shown that in the long term RIL might not be sustainable in relation to genetics, demography, and timber production. For example, the general RIL guidelines have been shown to be unsustainable for valuable timber trees from the Amazon, such as *B. guianensis*, *H. courbaril*, *Manilkara huberi* (SEBBENN et al., 2008; SILVA et al., 2008; LACERDA;NIMMO; SEBBENN, 2013), *Dipteryx odorata* (VINSON et al., 2015a,b), *Handroanthus serratifolius* and *Handroanthus impetiginosus* (SCHULZE et al., 2008b), among others species studied by Schulze et al. (2008a). Considering current logging standards used in Brazil, *Symphonia globulifera* requires a CC of 40 years; nonetheless *B. guianensis*, *H. courbaril*, and *M. huberi* require more than 100 years recovering to reach their pre-logging basal area, as presented in the in Sebbenn et al. (2008) (Figure 1). To achieve sustainable timber production for *D. odorata*, an increase of the MCD to 100 cm under a 30-year CC is necessary (VINSON et al., 2015a). Peña-Claros et al. (2008) also pointed out the need for the deployment of silvicultural treatments in order to increase growth rates of tropical trees. The study carried out in Bolivia showed that the growth rates of commercial species were 50 to 60% higher in plots that received silvicultural treatments than in the normal logging and control plots. Thereby, studies have shown that different species respond differently to logging, both demographically and genetically, requiring species-specific harvesting regulations to be sustainable in the long term (SEBBENN et al., 2008).

Figure 1- Basal area (m^2ha^{-1}) recovery for four tree species (a: *Bagassa guianensis* and *Symphonia globulifera*; b: *Manilkara huberi* and *Hymenaea courbaril*) after a single episode of logging, with 90% of individuals above diameter at breast height (dbh) of 45 cm (55 cm for *Hymenaea*) extracted.



Source: Adapted from the figure 3 from Sebbenn et al. (2008).

Post-logging regeneration in natural forests depends on mating among the remaining trees; however, in the RIL system the MCD does not consider the age at which trees become reproductive, and it is a crucial factor for some species whom become reproductive at sizes larger than the standard MCD (SEBBENN et al., 2008). For example, flowering of *H. courbaril* begins when trees reach a diameter at breast height (dbh) of approximately 49 cm while the MCD for harvesting is 50 cm dbh, thus leaving a limited number of reproductive trees after logging (LACERDA; NIMMO; SEBBENN, 2013). This leads to a decrease in seed production and regeneration, as well as significant decrease on the population' genetic

diversity (LACERDA; KANASHIRO; SEBBENN, 2008b; SEBBENN et al., 2008; LACERDA; NIMMO; SEBBENN, 2013). Reductions to the seed set after logging may also be the result of a combination of factors, including a lack of pollination and increased inbreeding as a consequence of restricted pollinator movement or a loss of alleles responsible for self-incompatibility (CLOUTIER et al., 2007; CARNEIRO et al., 2011). Because tropical tree species are expected to carry a high genetic load, inbreeding may produce inbreeding depression, resulting in the loss of adaptive traits, reduced vigour and fertility, poor stem forms, among other outcomes (LOWE et al., 2005). Through successive logging cycles, the composition of timber species within stands also moves towards softwood species rather than hardwood, with pioneer species becoming increasingly prevalent in the stands (PHILLIPS et al., 2004; VAN GARDINGEN, VALLE; THOMPSON, 2006).

3.4 DYSGENIC SELECTION

Sustainable timber production is directly related to regeneration in volume and quality; if individuals responsible for producing future populations exhibit desirable phenotypic traits (growth vigor, good stem form, pathogen and disease resistance, etc.), the subsequent generations are likely to either maintain or improve these traits, possibly increasing productivity; a technique known as tree improvement. Through the combination of tree improvement and silvicultural research (spacing, fertilization, irrigation, harvesting, etc.), Brazil has become a benchmark in the silviculture of exotic tree species such as *Pinus* sp. and *Eucalyptus* sp. Nevertheless, the forest management that is occurring in the Brazilian Amazon is the complete opposite. By applying a high LI combined with a MCD that is too low and CC that are too short, there is a significant risk of accumulating trees with low growth rate and poor stem form (dysgenic selection); thus, possibly leading to deterioration of tree populations due to the poor quality trees constitute a larger proportion of residual population, as has been observed in the tropical trees *Swietenia macrophylla* and *Swietenia mahogany* (NAMKOONG et al., 2002; CORNELIUS et al., 2005; VAN GARDINGEN; VALLE; THOMPSON, 2006).

Studies indicate that the genetic control of stem form is greater than for other growth traits such as dbh, height, and consequently volume. With a coefficient of heritability ranging generally from 0.3 to 0.6, stem form is more affected by logging, and two or three generations

of phenotypic selection might be sufficient to improve or deteriorate this trait (ZOBEL; TALBERT, 1984).

In Brazil, trees are classified into three stem form classes based on the length of commercial quality timber: 1 represents a tree with full length; 2 is a tree of at least one commercial log; and 3 is a tree with insufficient length for commercial quality timber (LACERDA; NIMMO; SEBBENN, 2013). Normally, 90% of all class 1 trees and a portion of class 2 trees are harvested, while all trees with poor stem form (class 3) remain in the population after logging (LACERDA; NIMMO; SEBBENN, 2013). Hence, it would be prudent to maintain most of class 1 trees in the population and remove only class 2 trees to improve timber quality and productivity for the future. Furthermore, there is a need to not only help the forest to recover (e.g. replanting the logged species), but also to maintain the landscape integrity around the exploited area, which would support migration of pollen and seeds into the area, avoiding long-term losses in genetic diversity.

3.5 ILLEGAL TIMBER TRADE

Illegal logging and trade of timber and wood products place legally logged products at an economic disadvantage, while also producing ecological problems, such as deforestation in timber producing countries (DEGEN et al. 2013). While, international laws and regulations, such as Convention on International Trade in Endangered Species of Wild Flora and Fauna, EU Timber Regulation, US Lacey Act, among others are in place, forged documents (phytosanitary certificates, invoices, and certificates of origin) are commonly used in the tropics to turn illegally logged timber into legal. Some anatomical methods have been used to identify tree species, but these methods may not be accurate enough to differentiate all tree species, particularly for difficult to differentiate species such as those in the *Handroanthus* genus, or detect timber origin. Recent developments in genetic based methods (population genetics/phylogeography, DNA barcoding and DNA fingerprinting) and chemical methods (mass spectrometry, near infrared spectroscopy and stable isotopes) to distinguish tree species and their origin have been proposed due to their reliability and high accuracy (LOWE; CROSS, 2011; JOLIVET; DEGEN, 2012; DORMONTT et al., 2015). The integration of these methods can be used to forensic timber identification to answer specific questions such as country or even concession of origin, genus and species, identify individuals and determine

age (DORMONTT et al., 2015). These methods may provide national and international authorities with an efficient and trustworthy tool to impede the illegal timber trade and promote trade with legally sourced timber and timber products, which would also contribute to forest conservation.

3.6 CONCLUSIONS

As discussed herein, actual logging practices used in Brazil are insufficient and inadequate, leading to the unsustainable use of natural timber resources, the impacts caused by illegal logging activities are even worse. Preserving the population structure, demography, and genetic diversity in logged tree populations is crucial to protect both forest ecosystems and sustainable timber production in tropical forests. Stricter measures to protect our forests must be applied, including the development of species-specific rules and regulations based on solid scientific studies for real sustainable forest management, integrated with population genetics and silvicultural knowledge and practices. Furthermore, we must favour the selection of the best trees for stem form and grow rate as the parent population of subsequent generations. Finally, putting in place reliable methods to control illegally harvested timber trade is the key to effectively confronting issues such as rapid deforestation and deterioration of the Amazon and other natural forests.

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4 CHAPTER 2: DEVELOPMENT OF A SET OF SNP MARKERS FOR POPULATION GENETICS STUDIES OF IPÊ (*Handroanthus* sp.), A VALUABLE TREE GENUS FROM LATIN AMERICA

ABSTRACT

A combination of restriction associated DNA sequencing (RADSeq) and low coverage MiSeq genome sequencing resulted in the identification of 402 putative loci (single nucleotide polymorphisms –SNPs; and insertion/deletions- INDELs) for Ipe (*Handroanthus* sp.). The full list of 402 markers is intended to help population genetics, phylogeography and DNA fingerprinting studies of this important and valuable tropical genus. The 402 SNPs and INDELs were successfully amplified in 94 individuals of *Handroanthus* sp. from Brazil, Bolivia and French Guyana, using MassARRAY® iPLEX™ genotyping.

Keywords: *Handroanthus* sp. Single nucleotide polymorphism. MassARRAY. Marker development.

4.1 INTRODUCTION

The genus *Handroanthus* comprises tree species commonly named as “Ipe” or “Lapacho” and belongs to the botanical family Bignoniacaea. Ipe timber is currently one of the most valuable timber species from South America, mainly used for flooring, decks or even for decorative veneer and naval construction. Due to its high-quality wood and high commercial value, Ipe has been overexploited. Schulze et al. (2008) evaluated the impact of logging in the Brazilian Amazon and concluded that *H. impetiginosus* ([Martius ex A. P. de Candolle] J.R. Mattos) and *H. serratifolius* ([Vahl] S. Grose)) are threatened due to the high intensity of exploitation, and that protection measures must be implemented.

This work intended to create an useful set of SNPs markers for phylogeography purposes and population genetic studies of this remarkable tropical genus.

4.2 MATERIALS AND METHODS

4.2.1 Sampling and DNA isolation

Handroanthus tissue material (leaf, cambium or bark) were collected in Bolivia, Brazil and French Guyana. The sampled materials were dried with Silica Gel and either sent to the Thünen Institute (Germany) or the Laboratory of Population Genetics and Forestry of São Paulo State University (UNESP), School of Engineering, Ilha Solteira-Brazil, for DNA isolation.

DNA isolation from leaf, cambium or bark was carried out according to Dumolin et al. (1995).

4.2.2 Marker development and marker screening

SNP/INDELs markers identification used in total five individuals identified as *H. serratifolius* (Table 1). Nuclear SNPs detection was based on restriction associated DNA sequencing (RADseq) (MILLER et al., 2007) and low coverage MiSeq genome sequencing for the detection of chloroplast and mitochondrial SNPs and INDELs (STRAUB et al., 2012).

A first set of 402 SNPs/Indels markers (Table 2) (15 chloroplast, 44 mitochondrial and 325 RADseq generated SNP markers [nuclear SNPs], 12 mitochondrial and six chloroplast INDEL markers) were identified according to sequencing data quality, different allele distribution patterns, and suitability for a MassARRAY ® iPLEX™ design (Assay Design Suite v2.0 [Agena Bioscience™, San Diego, USA]). A first marker screening was performed for all 402 loci using leaf and cambium samples from 94 *Handroanthus* individuals from Bolivia, Brazil and French Guyana (Table 3). Genotyping was conducted using the MassARRAY® iPLEX™ platform (Agena Bioscience™, San Diego, USA), using the iPLEX™ GOLD chemistry. Allele calling was conducted with Typer Viewer v.4.0.24.71 (Agena Bioscience™, San Diego, USA).

Table 1- Location and number of individuals used for SNP discovery from both RADseq and MiSeq sequencing approaches.

Sample code	Sequencing Technic	Country	Location	Latitude	Longitude
TASER_1	MiSeq	French Guyana	Paracou	5.26024	-52.93566
TASER_2	RADseq	French Guyana	Paracou	5.26001	-52.93647
TASER_40	MiSeq/ RADseq	Bolivia	Yapacaní, Naranjal	-17.43223	-63.92747
TASER_63	MiSeq/ RADseq	French Guyana	Kourou-Cayenne	5.06545	-52.55594
TASER_66	MiSeq	French Guyana	Kourou-Cayenne	4.98347	-52.44759

Source: author of this study.

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continue)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000004	T/C	ACGTTGGATGGCTGTAGGTCAAAGGAAATT	ACGTTGGATGGAATAAGCTACATGAGTACAC	ggtgTTCAAATGTGTAAGAATGGT
tas0000005	C/A	ACGTTGGATGATTAACCGGCGGAGGATTTG	ACGTTGGATGCGCTTCAGATTTAGCTTCGC	ggtctCTTCATGGTTCCGTAAAAAAGTC
tas0000013	A/G	ACGTTGGATGCATTTTTCTTAGTCCCCTTGC	ACGTTGGATGAATACGTCAGTCTAAGAAGG	CCCCTTGCTTCCTATG
tas0000023	G/T	ACGTTGGATGCGCAGGTGATTGAAAAATCC	ACGTTGGATGGCAGAGAATGGCACATTTTG	gagtAAGTATTGTCAATGAGGAGAA
tas0000026	A/G	ACGTTGGATGATAGAGCCTTTAATACCTCG	ACGTTGGATGTGTTTTTCCAGCCCCAGTTG	ccctcGCCTTTAATACCTCGAAATTG
tas0000034	G/A	ACGTTGGATGCTGGAAGCATTCAACCTCTC	ACGTTGGATGGCAAACTGTACTGCTAAGGG	ggggTACACAGTTCTCATGCGG
tas0000036	A/G	ACGTTGGATGCACCAGTGACTGGTGTAATC	ACGTTGGATGTTGCCTTCTTGGACCAAATG	aggaACTGGTGTAATCCCTGTCA
tas0000038	C/T	ACGTTGGATGCCATTCCAAAAAGTGAATGTG	ACGTTGGATGGTAGATAGCAACTGCACAGG	gggaTGCACAGGCTCCTGGGACTGC
tas0000042	G/T	ACGTTGGATGTGAATCCATAAGCTTTCCCC	ACGTTGGATGAGAGAAACATCACTAATGGC	tcaCCACATTGTTTGACTTTTAG
tas0000049	C/T	ACGTTGGATGAGGAAAATAATGGAAAGGG	ACGTTGGATGACGGAGTACTTAGAAGCTGG	tCTGGTTGATGTGAGGT
tas0000053	A/G	ACGTTGGATGTGAATCGACTATAGTCCCGC	ACGTTGGATGAGTTGGAGGGAAGAAAGTTG	CCGCCGTTTCCATTAG
tas0000056	A/G	ACGTTGGATGGGCAGTAAAATAATCTAGAG	ACGTTGGATGCCCTTTGGTTGAAAAGAATC	ttgtcAAATAATCTAGAGATGCATCATA
tas0000058	T/A	ACGTTGGATGCGACAGGTGATTAGTAGTAG	ACGTTGGATGGAACCTTTTCCTTTCCCTGC	ATTAGTAGTAGTATTATAGGATAGTGC
tas0000064	T/C	ACGTTGGATGGCCAACTCAATCCTTGTAAG	ACGTTGGATGGTGCATTAAATAGAATCAACC	TACAACTTATTTGTCATACGG
tas0000069	T/A	ACGTTGGATGAACAAGCAAGAATGACAGGG	ACGTTGGATGGTACTGTGAAATTATTTATTA	cggtGGGTACTGTGAGATTGTT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000082	A/G	ACGTTGGATGCCAATTACTTTAGGTAGGTG	ACGTTGGATGCAGAAGCATCATCAGTGACC	cggcCTTTAGGTAGGTGACTGGAA
tas0000085	C/T	ACGTTGGATGTCGGAACTGGTTCAGAGTTG	ACGTTGGATGGACAACTCTAACTGCCTTGC	TTCAGAGTTGGAAATTGC
tas0000097	C/T	ACGTTGGATGTCATGGCAAGGCCATGCATC	ACGTTGGATGGCCCATATCAAAAAGCAACC	CCATGCATCTCTTTTTTCA
tas0000104	C/G	ACGTTGGATGAAACAAAGGGATAATTGGGG	ACGTTGGATGCAACTGAACTAATAGTATGGG	cATGGGGTCTTAACGGGG
tas0000106	G/A/C	ACGTTGGATGCCCACAAGTTCTCTTACCAG	ACGTTGGATGGTTTGGGAGGATAAAGATGC	TTTTTTCGTTGTTTTTTCTCT
tas0000116	A/C/G	ACGTTGGATGTACGTGCTTGCAGTTTCCTC	ACGTTGGATGCAGTTGATGAAGCCATAAAAC	gcagAAAACATAGTATCAGAGTGC
tas0000119	A/G	ACGTTGGATGCACTATAGAAGTGCTTGTGC	ACGTTGGATGGCATCACTACACCTTGCAAC	tggcGTGCTTGTGCTTATTTAGC
tas0000128	A/G	ACGTTGGATGCATTCATTCTGCTTTGGGTG	ACGTTGGATGGGATGGTCTCTTTACATGCC	tttTTTGGGTGTTTATGATTCTTT
tas0000129	G/A/C	ACGTTGGATGTGTACCATCCTGACAGTGAC	ACGTTGGATGAGAGAGAATATTCAGTCAGG	CAGGACTCAAGAAATGCTGTA
tas0000131	G/A	ACGTTGGATGCCTTCCCGCCATAATGAAAC	ACGTTGGATGGATGTTGAATGAGAAAGAAG	tgacACTTATCATTGTATCTCTCACG
tas0000134	A/C	ACGTTGGATGAGATGACAGCACAGAGTGAG	ACGTTGGATGTACATTTTGTGTTTTCATAG	ccacTTGGCTCGAAACTGAAACTA
tas0000139	T/C	ACGTTGGATGGACCGAGCTTGACATTGATG	ACGTTGGATGACGACTTGGAAAAGGTCGAG	ataCCTAGTCTCGCAGAAGT
tas0000144	G/A	ACGTTGGATGCGCGTCGAAGAGTTTCGAGA	ACGTTGGATGTGCAAACTCATCGCCAAGTC	GCTCGGCTCGCCTTC
tas0000145	G/A	ACGTTGGATGCAACATGTCCCTGAAATCCC	ACGTTGGATGGGCTTAATATCTCGATGTAGG	GAATAAACTCAACCCGATTAA
tas0000151	T/C	ACGTTGGATGAATGCGGGAATTAACGGTGG	ACGTTGGATGGGGTAGCCACTTATCCCATA	gagtTGGGGGTGGCCTACTTTCTCAG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000155	T/C	ACGTTGGATGCTAGCATTTCTAAGTTCATC	ACGTTGGATGTTTCGAGCTCCAAATGTGCC	ccctTGCCTCGCTCATCATA
tas0000160	T/A	ACGTTGGATGAAGCAGATAGACCTCGAGAC	ACGTTGGATGTCTCATCCTCGTCACTTTGG	ccagcCTTTTCTAGAGAAGGCATATC
tas0000163	G/A	ACGTTGGATGAAGATATAAAGGTTAAAATAG	ACGTTGGATGGTTCTTGTTTTTTGGATGG	GGATAATTGCACGCCT
tas0000164	A/G	ACGTTGGATGAGCACATAGTCAGCAGCTTC	ACGTTGGATGCAGCTGCAGATGTAGGTATG	GGCAATAGGTGCAGG
tas0000165	A/G	ACGTTGGATGATGGCCACATGATCCCAATC	ACGTTGGATGGGAGTGGAAATGATGGTTGC	ccctTAGACATGGCCAAACTATTC
tas0000166	A/G	ACGTTGGATGGCACCAGTCCAATATAAAATG	ACGTTGGATGGTTGTTGTCAAGGTGAATT	TTACTTTTTGTCGTGTGG
tas0000172	A/G	ACGTTGGATGGAATCCCCTGTTTCACTCTC	ACGTTGGATGCTCAGCTCCAACATCTTTAC	ggcatCTCCAACATCTTTACTCAGTGAA
tas0000176	G/A	ACGTTGGATGTTCTTCACCAGGCTTGTGAC	ACGTTGGATGGAGAACACCCACATGGTAAG	ccccAGTTATGTTGCCTTTAGTTT
tas0000181	G/C	ACGTTGGATGAAGATGATACAAATCAGCCC	ACGTTGGATGAAGGTCTTATAGTTAGGAGG	gCTTATAGTTAGGAGGATAGTCATC
tas0000189	A/C	ACGTTGGATGCTTATGAATAAATCCAATCCC	ACGTTGGATGGGCTCCATTATTGGTTCTTG	TCCAATCCCATTTATCTTGA
tas0000209	C/T	ACGTTGGATGTTCTCAATTGCTGGTGGTCC	ACGTTGGATGACCCCGCTGGTTTATATTGG	ACCATGTCTAATGGAAATTC
tas0000211	T/G	ACGTTGGATGAACCCCAACAGGTGACAATC	ACGTTGGATGGCTAACAATATTTCAAATGC	ctaaaAATATTTCAAATGCTTCTCAAA
tas0000219	A/G	ACGTTGGATGTTTGCATCGGTAACAGCCAG	ACGTTGGATGAGTGGGTCCAGAATCAGTTG	ATCAGTTGGTGCACA
tas0000226	A/G	ACGTTGGATGGGTCTTGAGTAGCCGTTTTC	ACGTTGGATGACAGGCTTCTTAGTGGACAG	cttcCTAGGTATCACCACTTGA
tas0000228	A/G	ACGTTGGATGTGTGGCTTTAAAGAGATTCC	ACGTTGGATGAGAAGAGCTTATGGGACGAG	taggtGGCTTTGGAGCTGCC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000229	C/T	ACGTTGGATGGTTCCCTTAACCTTCCAACC	ACGTTGGATGTGAGCAACTATGCAGACTTC	gggttCAGACTTCAGTTGAAACTTTCT
tas0000244	G/A	ACGTTGGATGAGCTTTGGTTGGACAGAGTG	ACGTTGGATGACTGAGACTTCACCAGCTTG	aTGGGAGTGGAAAATCTACC
tas0000247	A/G	ACGTTGGATGAAAGCCCCCAAGATTCACTC	ACGTTGGATGCAGGAACCTTATCGATGCTC	GAAGCAGCAGCTGAC
tas0000253	C/G	ACGTTGGATGATTGTCAGCAAGCATCAGGG	ACGTTGGATGTGGATGTGCTTTCCTCTCAG	cAGTGTCCATTACTTGCT
tas0000258	T/C	ACGTTGGATGGGGGTAATAAAAGACATGTG	ACGTTGGATGGAGGCATTCAGTTTATTGGC	ggGTGAACAGATTAAATGTGCTG
tas0000259	T/C	ACGTTGGATGAACGTATGGAATGTCCTCCC	ACGTTGGATGAATCATTTCCACTGGCTCGG	cTGTCCTCCCAAAATGC
tas0000262	T/C	ACGTTGGATGTAGAGTGTAGACTCCCCTTG	ACGTTGGATGATCTACCAAGGTTTGCCGTC	gTTAGTCATTGAAATGTAAAAGGATA
tas0000266	T/C	ACGTTGGATGTATCAACACGGGAAACTGAC	ACGTTGGATGCTTCCTTGATGATTTGACTGG	AACTGACATTACATTACCTC
tas0000270	G/A	ACGTTGGATGCACCATGAAACACCATCCAG	ACGTTGGATGGGGTTCTGACCAGTTAAATC	aTGCAGCAAACAAAGC
tas0000279	G/A	ACGTTGGATGGCTTTTCAAGTCATGATAC	ACGTTGGATGCATCATTTACTATAATCAAG	ggggtTATCAATGTGAATATAATGGCAT
tas0000290	G/A	ACGTTGGATGAACTGGTATCTCCATCTGGG	ACGTTGGATGGCTTACCTGCTGCATGATTC	GGATTATTCAAATGATAACCAAAC
tas0000294	G/A	ACGTTGGATGGCCCAATTTTCTGGATTCTC	ACGTTGGATGTACGGACATGTGGAGACTGC	ccACTGCCCGTATTGTCG
tas0000296	C/A	ACGTTGGATGTGCCAATTACAACTATGGAG	ACGTTGGATGGACAGGTTTGATTTGATTTTG	gggaATTTGATTTTGATATATACACGGC
tas0000300	T/A	ACGTTGGATGCGAGTAAGCTGATTTATCTTC	ACGTTGGATGCAAGTATCCCCGAACGAATC	aCCGAACGAATCATTGGTC
tas0000326	A/G	ACGTTGGATGGTGGTATTCATACAGCTTGG	ACGTTGGATGAAAAAGCAACCAACATGAG	gtttGAAGCAAGTTAAAGACAATG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000329	A/G	ACGTTGGATGCATGGGAAACTTCTGTTCGC	ACGTTGGATGTTACCTGATGCTTGAGTAAG	cccgGTTCGCATCCATGCAATCTTTC
tas0000340	A/G	ACGTTGGATGTATGTTTCTTAGTGGCCAGC	ACGTTGGATGGTGCATGGCCCAATAATTCC	acTAATTCCTTGGATCTTTATATCTTT
tas0000345	T/A	ACGTTGGATGTATTGAGAAGAGAGGACCCC	ACGTTGGATGATAAGGGACTCCTCTGTTGC	ccCCTCTGTTGCAGTCAT
tas0000347	A/G	ACGTTGGATGACTCCATTCTTTGAGCCCAC	ACGTTGGATGCCCCCAATCTTGCATTGAAA	ccaACCTGTAATTCATAGAGATATAAA
tas0000358	A/C	ACGTTGGATGGATCTCCAATTTCACAATGGC	ACGTTGGATGTGGTATCTGCTGCTGTTTAC	gatgTCACAATGGCGGTGAG
tas0000360	C/T	ACGTTGGATGAATGGCCCTTTCTTTGGCTG	ACGTTGGATGTTCGCCGATCCATTCTTCTC	ccccaTTTGGCTGAGGCAAAATT
tas0000364	T/G	ACGTTGGATGGCAGAAATCCGAACCATCTC	ACGTTGGATGAGCAGAGAAGCTTATTGCTG	aTCTTGTCTCTGCTTCC
tas0000370	G/A	ACGTTGGATGCCCTGCTTAATGAAGGTGAG	ACGTTGGATGAAGCATCAAGTGTTGCACAT	atggGAGACACATGAATTGAACAGTT
tas0000380	T/C	ACGTTGGATGCTAGTACCCTCCTTTCCCAC	ACGTTGGATGGGAAGGCGTTTAATCATTC	atcTAATAAATTATGCAGCTGAAGAAC
tas0000381	C/T	ACGTTGGATGGTTTGCTCATGCTTGTATGC	ACGTTGGATGGCTGCTGCTTGAGAAACTTC	cACTTCTCCCCCTAATGC
tas0000383	G/A	ACGTTGGATGCCGAAGCACTTATTCGTACT	ACGTTGGATGTGATTCAGATTCTTGCTTG	TTGCTTGTAATAGTTTGATATATACATA
tas0000394	G/A	ACGTTGGATGTGAAGTGTAACTGGCTTGCG	ACGTTGGATGCCCCAACCTACAAAAAATG	ccacAAATGAAAGAACTACCGC
tas0000400	A/T	ACGTTGGATGGAAGATTGGAAGTCATTTAC	ACGTTGGATGTGTTCGATTTGGTATAAAG	agggtAAGTCATTTACACCCTATTTT
tas0000416	A/G	ACGTTGGATGTGGCCCCTTTCATTTAGCAG	ACGTTGGATGTTAAGTACCTTGTCCCAGCC	aacacTTAGCAGACAACTTGTCACTTAT
tas0000431	G/A	ACGTTGGATGGTCTGTATAATGCATTGGTG	ACGTTGGATGGTCAAGCAGCTCTACAAAAT	AATGCATTGGTGATTTCAGATTAATA

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000443	T/G	ACGTTGGATGGAGACCAGGGAAAATTAATTG	ACGTTGGATGCCCCTTTTTTGGCTTGTATG	aaggTCAAAGCTTCCATTACCTA
tas0000454	C/A/G	ACGTTGGATGTCCAAGCAATGCCCTAATAG	ACGTTGGATGCGGAGTTGGACCTTTTTAGC	cctcaAAGCAATGCCCTAATAGAGGGAC
tas0000457	C/G	ACGTTGGATGCCTACAAGGAATGATATGCG	ACGTTGGATGGCAGATGATTTAGATAGTACG	gtCGTGAATTTATAAAACATGCAG
tas0000459	C/T	ACGTTGGATGTCGCATGCCTATAGAATGCC	ACGTTGGATGGCCAGGAAACAGAACTCATC	CCAGGTGTGATTCTGG
tas0000464	C/T	ACGTTGGATGGGTTGGGTGATATAATGGAG	ACGTTGGATGTTGACCTGTCTAGAGTCTGG	ggggtTCTAGAGTCTGGGATGAC
tas0000482	T/C	ACGTTGGATGCCCTTCTTCCTGAAACAATC	ACGTTGGATGGAAGGTAATCATCTCGATTGG	ggacATCTAAAGTTTAGTGCTGAATG
tas0000483	A/G	ACGTTGGATGCCTTTGGTTGAAGCAAGAGG	ACGTTGGATGAGCTGCTCGTGAGAATTTCC	CGTGAGAATTTCCTCATCAG
tas0000485	T/C	ACGTTGGATGATCCGTTGCGCCAAAGAAAG	ACGTTGGATGGACACATACGATAGTCCCTC	ACATGCAAATTAGAACTCGAAG
tas0000486	G/C	ACGTTGGATGTACAAAAGGGACGGATGAGG	ACGTTGGATGCCACACATTGCAGTGTTATC	TGAGGCAGAGGAAGT
tas0000491	C/G	ACGTTGGATGTATTGAGGATGACGGGGAAG	ACGTTGGATGCCAGTGAAGTATCTACACAT	ttatcCATCCTCAAATACAACACT
tas0000496	G/A	ACGTTGGATGCAGAACAGAAGTGAAAAGGC	ACGTTGGATGTGGTGAACACTACATCGCAG	CACAAATAACTAATGGGTTCA
tas0000497	A/G	ACGTTGGATGGAAGTCAAATAAACCCCAGTC	ACGTTGGATGCAAGGACATAATTCTCGAGG	ggCGAGGTTATATCATCTACATTTG
tas0000500	C/T	ACGTTGGATGTGTGGTAAGGGCTGCTTATG	ACGTTGGATGGTTGCACATTACTTTAATCG	tgaTTATGAGTGATCTTGGCTTTTCA
tas0000503	G/C	ACGTTGGATGATCCAACGGTCTTGGTAAGG	ACGTTGGATGTTCAGTTCGCTTTCTCTTGG	AGGCTGCAGCATTAAG
tas0000504	A/C	ACGTTGGATGCCAACTAGACCTGCTGCAAG	ACGTTGGATGTGGGTATAGATGGTGGTTGG	GGGGCTGTGAAAGAA

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000513	A/G	ACGTTGGATGGTTAATATTGGGTATCCCTG	ACGTTGGATGCCTCTCCCATCATCATCAAC	cccgAGTAACGTGCAGCATGGTTCTAG
tas0000515	T/A	ACGTTGGATGGTTAGCACCTGTATATGAG	ACGTTGGATGTGTAATTGCTTGGGCTGGTG	gttGGCTGGTGATGATGTT
tas0000519	G/C	ACGTTGGATGATCTCAATGACGAAATATC	ACGTTGGATGAAATCTCAACGTGTTCGACC	GTTGACTTTTGAATGTTAACAAATC
tas0000527	G/A	ACGTTGGATGAGATGAGTCTGGACCAAAAG	ACGTTGGATGCGATGCTCCAAATGGATTTC	taggTGAGTCTGGACCAAAAGATAGAAC
tas0000535	A/G	ACGTTGGATGGTTAAGGCTCCATAACTGTG	ACGTTGGATGTCCAAGGTTCTGTGGATTTC	GGAAATGGACTATCACTCTA
tas0000541	A/G	ACGTTGGATGATCAGATCATCTGTGTTGGC	ACGTTGGATGCGTGTGTGCTTCTTTCTCAG	gggTCCTGGGATACTGGAA
tas0000546	T/C	ACGTTGGATGTGCTGCGGAACTTTGTAGAC	ACGTTGGATGCAGCAGGGAGACAAATATTG	ggTAAGTGGGAAATAACTGCTT
tas0000548	C/T	ACGTTGGATGGGTGAAGCAAATAGGTTGAC	ACGTTGGATGGTAGCTTATGCAGCAGACAG	ATAGGTTGACAAAGTTGCT
tas0000556	T/C	ACGTTGGATGATAGGGTTCGATTCAGTTGC	ACGTTGGATGGCATAAAACTGAAGAGTTAC	cCCACATACCTCTTCGGC
tas0000559	T/C	ACGTTGGATGTTCGATAATCACAAATTGC	ACGTTGGATGATTTTGACGTTTGGCGCTGG	tcCAATTTTCGATATCTTCAAAATACA
tas0000565	A/C	ACGTTGGATGCAGCAACCAATTCAGCAAGG	ACGTTGGATGCCCACTAATTTTTCTTTTG	cccAAAACACATTATCAACTTCAAT
tas0000571	T/C	ACGTTGGATGGTAGCCCCTCGAGAAGAATC	ACGTTGGATGTGAGGCTGCGGATAATTTTG	cccgCGAGAAGAATCGGGCTCCTAT
tas0000576	A/G	ACGTTGGATGTTTTCTGCAAAGGACCCCAC	ACGTTGGATGACCGTTCAACAGTTAGCTCC	ctgcCCCCACTTGGAATCC
tas0000587	A/C	ACGTTGGATGTTGTTAAAGAGCATTGAGC	ACGTTGGATGTGCAAAGTCCAAGCACAAGC	gaagGCATGAGCTATGTTTATTTGG
tas0000588	T/C	ACGTTGGATGATACACATTGACACCTTAG	ACGTTGGATGTTACAAGGTCCTTCAAGATG	ACACCTTAGTGATCTTGAG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000598	T/A	ACGTTGGATGTTCTCCAGGAACCAGCTAAC	ACGTTGGATGGCTTGTTTCTTGAGAAGTCC	gcacAACAGTGAAGTTTAAGTAACCTAA
tas0000606	C/T	ACGTTGGATGAAAGATTAAACCACTGAGC	ACGTTGGATGACCGGCAGAGATTCTATTAC	TCTATTACATCAATCTAGAAATTTAAAC
tas0000616	A/C	ACGTTGGATGTGTGGCATCCAGCATGATTC	ACGTTGGATGGATCAACACTGACCCGAAAC	gggGACGGATCATCCGGTG
tas0000622	T/G	ACGTTGGATGGGAGTTTAAAGCTATGCAAC	ACGTTGGATGTCCCGATATTTCTTCAGAAC	gggaTATGCAACATCTGATATTATCTTT
tas0000633	G/C	ACGTTGGATGGACATCCCAAAGTCGAAAAG	ACGTTGGATGTGTCGAAGGAGTAGAAGATG	tcaacGTCGAAAAGATTCTTGTCCA
tas0000637	G/A	ACGTTGGATGCATAGTTGTTGATGGTGGAG	ACGTTGGATGTATTGAAGGCAAAGTCGCAC	GCACATTCAGATGCCG
tas0000644	G/A	ACGTTGGATGCCAACCCCACGTCATTATTG	ACGTTGGATGCATATCTAGTTCTATTGAC	acaATCCGTTTTCTAGAACAATC
tas0000656	T/C	ACGTTGGATGGTTCTCAAAGCAACTAATC	ACGTTGGATGTAGGAAGGAGCTAGGAAAAC	CAACTAATCTTTAATCAAGTAGCAA
tas0000660	C/T	ACGTTGGATGGGTCAAGCAAATTCTTCACTG	ACGTTGGATGCAATGGCATCATAGTACGGG	cgggTCGTGCTTTCAAAGGG
tas0000661	A/G	ACGTTGGATGTACAAGTTGCCACTCCTCTG	ACGTTGGATGTTCTAGCTGGATGTACAGAT	AAACTAGATCTGTTGAATCAGAC
tas0000666	T/A	ACGTTGGATGAGTTACTTCATCTGAGTGAG	ACGTTGGATGAATCGCCTAAGCTTCGAGAG	taagaTTCGAGAGTTTGCCTAT
tas0000676	C/T	ACGTTGGATGGCAGACCAGTCCTTAATTAG	ACGTTGGATGGGATGAAGGACGCTTTTGTG	TCAGATGTTCATTAAATACAGTAA
tas0000681	C/T	ACGTTGGATGGCAAACAAAATGATTTCCAGG	ACGTTGGATGATTGGACATCCTTTGGGTGC	ggcgTTGGGTGCAACAGGT
tas0000684	G/A	ACGTTGGATGCGTATAGAATGACTCCGGTG	ACGTTGGATGTGTTAGGCGTGTGATGCTAC	gtgAGGCGTGTGATGCTACAGACAAA
tas0000687	T/C	ACGTTGGATGTCCTTTGCATTCCTGCTGTC	ACGTTGGATGCAGCAATAGAAGTTCGGCAG	CGGCAGTTATCACTGCAC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000690	A/G	ACGTTGGATGAATCTGCTTACAGGCCACTC	ACGTTGGATGTGGGAAGATCCCTTGTTACG	TGTTACGAAGGGAAAAGC
tas0000725	G/A	ACGTTGGATGTGATGATGGTGGTTGTCGTG	ACGTTGGATGTTGGTGGTCATGGGATTTGG	CCATGGTTACACTGGT
tas0000726	A/G	ACGTTGGATGAATCAAGCAGAGCTTGTGGG	ACGTTGGATGGCCACCTTCTTCCCATCAAC	cccctCTGCCAGGTTCCACTG
tas0000733	C/G	ACGTTGGATGCTTCCAAAATGAGGGCATCC	ACGTTGGATGGGGTTATGATGGCGGACTTG	cagaATCCAATCCAATATTCCCAA
tas0000735	G/A	ACGTTGGATGAACAAGTTCCCAAGATAGGC	ACGTTGGATGGCAGCCCTTAATGATCCTTC	gggcTTCCCAAGATAGGCTTTCTG
tas0000737	G/A	ACGTTGGATGGCTTTTAGACGAACCTAGAG	ACGTTGGATGAGCCACTGAATAAGGAGCTG	ctcATGCCAATATGGGTCCACT
tas0000749	C/T	ACGTTGGATGCTTGTTCTTAGCTGGCTCAC	ACGTTGGATGGGGCACCTTCCTGTTTTTAC	ttccgGTTTTTACCTGTATTGTTGAGTT
tas0000754	G/A	ACGTTGGATGATCTGATTGTTACAAAGGGC	ACGTTGGATGATCTCCGCCTATAGCATGTG	GGCAATTTCAGGGGG
tas0000759	C/A	ACGTTGGATGGCTACTTTTACCTACGGGTG	ACGTTGGATGTTCAAAACAGGGTCAACCGC	CGCACATTCCCACCC
tas0000766	G/A	ACGTTGGATGTTGATGTAGGAGCAGGTAGG	ACGTTGGATGAAGGAGCCAGTCCTTCTCTC	tgagGAATACAATTCCATGGCGTC
tas0000772	C/T	ACGTTGGATGAACAGGACAAGTTCTGCAAC	ACGTTGGATGAACATCCACTGATGGCTCTC	gGGCTCTCAGCCAAAA
tas0000794	G/A	ACGTTGGATGTTACCATCGCCAATGGCAAG	ACGTTGGATGGCTCCTTTGCAAAAAGCTGG	ggcagGTCATCAGTTCTTTTTTTCATCA
tas0000807	G/C	ACGTTGGATGCCCTGATCGTATTTTCCTCG	ACGTTGGATGCCTTTATGTTTTGGCCCCAG	CTCGTTCAAGTCCTCC
tas0000812	G/T	ACGTTGGATGCTGATCAATTTTTCAAGAG	ACGTTGGATGCTGAGACTCATAAATTCAAC	ggtaAAGAGTATGCTTACTGGG
tas0000813	T/C	ACGTTGGATGTAGCAATGCCATTAGGCCTG	ACGTTGGATGCCGTAATTCGAGTACTCACC	GGATGGCTAGAATGGAA

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000819	A/G	ACGTTGGATGTACTGCAAGCTTTTGGCAAG	ACGTTGGATGAAGGCAGATCACATGTGCAG	TTGTACACATTCTCTGAATTCTC
tas0000823	G/A	ACGTTGGATGGCTATTGACAACCTCAGCTA	ACGTTGGATGTCGAAGTTAATTGCTCTCTC	aaacCAACGAGAAATATGAGAAACC
tas0000832	C/A	ACGTTGGATGTGCAGCAGTGAAGAACTTGG	ACGTTGGATGGTGGATTGAAGAGTTGGAGC	ggtgGGAGCAGTTGATGCTCTT
tas0000835	G/A	ACGTTGGATGCCTAATTGCTGTTGGAGCTG	ACGTTGGATGAGACCCACCATTGATGGTTG	GTTGATCAAGAGACCTCA
tas0000836	C/T	ACGTTGGATGCAGTGAATCAACTTCTTGCC	ACGTTGGATGCGAAACATACTGAATAACCCG	ttacAACCCGACAAATTATTTACCA
tas0000838	T/C	ACGTTGGATGCCCAATGGAAAAGTTGCCAG	ACGTTGGATGTCTTTCGTCCTGACTAGGAT	gCAATGTTTGTAGCTTTCATATCTTC
tas0000846	A/G	ACGTTGGATGGGTAAATTCCAGTTTGTCGTC	ACGTTGGATGGGTAATCATTGCTATGAGCG	acTTCCAGTTTGTCGTCTATATATACAC
tas0000859	C/T	ACGTTGGATGTGGAGAGAGGTCTTATGCAC	ACGTTGGATGCCTGGATCTGGCTTAGACAC	GCCAGTAGAGATCAGTT
tas0000860	C/T	ACGTTGGATGGATTGCTGAATGCTGTAGAG	ACGTTGGATGACCATTTTGGCATGATTCTG	TGGTGCTGATGTGGT
tas0000862	C/A	ACGTTGGATGATTGTCATCCCGTGGTACAG	ACGTTGGATGCTTTCAGCAATTCAAACATGG	cccTTCAAACATGGAATCCCC
tas0000864	C/A	ACGTTGGATGTAGAAACAGGACACGCAACC	ACGTTGGATGTTTTGTTCCCTCCGGATGTG	cccccCTGAACCATCTGGGCT
tas0000865	A/G	ACGTTGGATGTCCTCTTTCTTACAGCCACC	ACGTTGGATGGAGTTCAACCTGATGTTCGG	CCACCCCATTCAGACTCTC
tas0000871	A/G	ACGTTGGATGTCCAGCGTTTATGGCATAGG	ACGTTGGATGGCAAGAATACTCGGATGCTG	ccCCGCCTCCGAGATCG
tas0000877	C/T	ACGTTGGATGTTGACCGTCGAAACTGACTC	ACGTTGGATGGGCAAGTTGCATTCATGCAG	gGGATGCTGTAATCTTCACTTTG
tas0000880	A/C	ACGTTGGATGTGATAAGTAGATAAGTTGG	ACGTTGGATGCACTGGATAGAAAAATGAG	gtTTTATATTAACTAACCACATCCAAT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000883	T/C	ACGTTGGATGAGATTACAGTACGCCCACAG	ACGTTGGATGCTTCTTACCTTGCTGCCTTG	CTTTCAACACGTGAAGG
tas0000886	C/A	ACGTTGGATGAGTTACTGAGGAGTGCCAAC	ACGTTGGATGCTCCCAACTGTGTATTGAAC	GCCAACAAAATAGTAACAGATAATCAT
tas0000888	C/T	ACGTTGGATGATCCTTTCTCGTTCTTTCCC	ACGTTGGATGAGTTGATGCATATAGTACGC	tccgATAGTACGCTCCTCCT
tas0000897	T/A	ACGTTGGATGACGCAGTGTGAACCCTTATC	ACGTTGGATGCTCACTGGGATTTCCATAGG	ACAAGGTGCGACTGG
tas0000898	T/G	ACGTTGGATGAGCAAGAAACTTTTTGCTC	ACGTTGGATGAAACTTACGACGCTCACTGC	GCAATCCCACTGCCC
tas0000912	A/G	ACGTTGGATGATCCAAAACCCACGTAGTGC	ACGTTGGATGCATGCTCATTATTGGCCGTG	ctagtCGTCGGGCTCGGTTT
tas0000919	T/A	ACGTTGGATGGGGCATCATCCTGTAAAACC	ACGTTGGATGCCCAGGTATTTTTGACTTTGC	ctcacACCATCACAAAGTAAATTAAATG
tas0000921	A/G	ACGTTGGATGTAATGAGCAAGCGCCTTCAC	ACGTTGGATGTATTGTCCATAGTTCCACCC	GTTACATTTGCTTTTTCAGGG
tas0000938	G/T	ACGTTGGATGAAGGCTTGGAAAAAACCCAC	ACGTTGGATGGGTAATTTGGAATGTGGTGC	gTGGCTTTTGTAAAATGGATG
tas0000946	T/A	ACGTTGGATGGCTCTGAAGCACTCATTTTC	ACGTTGGATGTCCCAAGTCCATGAGTCTCC	aggagTTTGTGTTCAGAATATGAAGAT
tas0000952	A/G	ACGTTGGATGAATGGGATGCAGATGCCAAC	ACGTTGGATGCTGGGAAGTTGTTCCAAGAA	ACAGTGATCCTATACCTAAAGT
tas0000956	A/T/C	ACGTTGGATGCTGCTTTTTTCTCATGATGC	ACGTTGGATGTCTTGAAGCACCACGTAGTC	cccgCCCCTCTGCAACCTTCA
tas0000957	T/G	ACGTTGGATGCTCAAATCCTGCTTTGCAAG	ACGTTGGATGGATAAACAAACCACCGCCAG	caggCCTAAGATGATGAAATCAACAC
tas0000965	G/T	ACGTTGGATGTTCTTCCCATCAGGACCTAC	ACGTTGGATGGTTTTCAATGTGGGCCAAGC	ccAGCATTAACAGTGTGCTTTTAT
tas0000984	G/C	ACGTTGGATGGGACAAAAGTATCATGATTGC	ACGTTGGATGCTGAGGTGATCACCACTTAC	gatgTCAAGAAAGATAGCACCAAT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0000993	G/A	ACGTTGGATGGGGCTTATGGGATGAATTTC	ACGTTGGATGGCCAGTAGTGGTATTCTTCC	TGCTTGGAGGAGACA
tas0000995	A/T	ACGTTGGATGCACACTGCAAGAAAATGATG	ACGTTGGATGCCATTTCCTTGAAATCTCACG	TGATGCGTCCACATT
tas0001017	A/G	ACGTTGGATGACGATTGTCTCATGTATAGC	ACGTTGGATGGGTGTCTGTATAAAGCTTTGG	gtgggGCTTTGGAAGAATCAGAGTA
tas0001032	A/G	ACGTTGGATGGCATGGAAAACGAACCGTTG	ACGTTGGATGGCTTGAAGCAAGTTATGCTG	ggGCTTGAGCATTTGACTG
tas0001047	A/T	ACGTTGGATGTTCACTGTCACGATTGGTCC	ACGTTGGATGTATGGTGAAAACGACACGGG	tagggTCGTGGTTGTTTAAGGAACAAT
tas0001048	G/C	ACGTTGGATGTGAGGAAGCCAAGAGGAATG	ACGTTGGATGACCCATAACCATTATCTGGC	tcaaACTCTGAATTCACACTTTTACA
tas0001052	T/G	ACGTTGGATGCCGTCATTGTTCACTATTAG	ACGTTGGATGGCAAATGAAGTATAACGAG	gcTAACGAGAAAATGGATAGCCTAT
tas0001054	G/A	ACGTTGGATGCAAGATGAGCAAAGGAGGAG	ACGTTGGATGAGTCGATAACGAACGTCACC	TCTTCTTCCCTCCCTT
tas0001055	T/C	ACGTTGGATGGGCCGTGAAGTTTACATTTG	ACGTTGGATGCAGGGGATCAAGCTCAAAAC	cGGAATTGGAATCGGGAG
tas0001064	T/G	ACGTTGGATGAGGTCCACTTGCGAACATC	ACGTTGGATGTGCAGAAAGAGTCTTGGCTG	TCCAAGTACCCAGAAAAAC
tas0001065	T/C	ACGTTGGATGCTCTGCATTAGACAGTACAC	ACGTTGGATGGTGTCATATCTTTAACTCAGC	gcCTCAGCTTCTTTCTTATCAAC
tas0001067	C/A	ACGTTGGATGGCAGAATACAGTTCCGACAG	ACGTTGGATGATGGCACAGCTTGGTTTATG	GCAACTATGCGCTTCAG
tas0001072	G/C	ACGTTGGATGGTTTGCCACATCTTCTTCTG	ACGTTGGATGGAAGTGGACAGGTTGAATGC	CCAATTTCTGCCTGCT
tas0001082	C/T	ACGTTGGATGCAAAACGGGTCGACTCATAG	ACGTTGGATGAAATGGAAATACCCAACCCG	gggagCATCAATGTTGGTGGGTTAAAC
tas0001083	G/C	ACGTTGGATGGCTTGCTTAGTTTTAATTTG	ACGTTGGATGCGGATATCTACATGGAGGAG	TCTATCAGTGAAAACCAAGG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001084	A/G	ACGTTGGATGTTCTCGGCTCTCTATCCTTG	ACGTTGGATGTAGCATAGCAGCTGCACAAG	GCAGCTGCACAAGTAGGTTG
tas0001088	A/C	ACGTTGGATGATTGAAAAGCCACCAGGGTC	ACGTTGGATGTTTTTCTAGCTGTGGAGCCG	cccaCGGAGTGTATTTATGCTGATGTTC
tas0001089	C/T	ACGTTGGATGAGGCATTGTTACGCATCAGC	ACGTTGGATGGGCGATCTTCACTCCAAAAC	tttaaGCATCAGCTTTATGTGAACTA
tas0001092	C/A	ACGTTGGATGCGAACATTGGAAAGAAGTGG	ACGTTGGATGCATTGGACCAGCTAATTCCC	gcAAGAAGTGGTTAGCAAATCATATA
tas0001098	C/T	ACGTTGGATGGCTTTGAAGCCTTGGATCTG	ACGTTGGATGCTTTCAGGCTCGACATTTGC	gTTTGCTAGGTGAGGC
tas0001103	G/A	ACGTTGGATGGCCAAACCTTAGCATTGTTG	ACGTTGGATGCAATTATATGAAACTGGCTC	GAAACTGGCTCCATTTG
tas0001110	A/C	ACGTTGGATGGAACACAAATGATGATCAG	ACGTTGGATGGTATGAGTTCATGTGTATGAG	TGTGTATGAGATTTCTTGTAGGTTAT
tas0001111	T/A	ACGTTGGATGAGTGAGCCAATTTAAGCATC	ACGTTGGATGGGTGTGAGTTGGCATTCAAT	GGCATTCAATGTACTGAAC
tas0001118	C/T	ACGTTGGATGCACGGATGTAGTAGAGATGC	ACGTTGGATGTGGTGGACAGTGCACCAAAC	gGAGATGCAGCAGGAATTT
tas0001119	C/T	ACGTTGGATGTACTGATGGTAGAGAGTCGG	ACGTTGGATGCCCAAGCTTTCATCATCGTG	CCTTGCTTGCAGCAG
tas0001123	C/G	ACGTTGGATGGCCCCATCAAAAGCAATGAC	ACGTTGGATGGGATTTGGAACTGTGAAGAG	tcatGCTAATCAGGCCCTC
tas0001127	A/G	ACGTTGGATGAGTCCTTGGAGGTGATGTTC	ACGTTGGATGCCCAATTCTTTTGCGAGCTG	ggggaGAGCTGCAATTTCCTTT
tas0001130	G/A	ACGTTGGATGCACCATTGTAATTGTGGTATG	ACGTTGGATGGAGGATAAATATGTTTGTG	GGATAAATATGTTTGTGCAAGTA
tas0001132	T/C	ACGTTGGATGAGGTAGGTAATAATGGTGAC	ACGTTGGATGCAGGTTATTGATGCTTGTTTG	AATAATGGTGACAGAGGA
tas0001135	A/G	ACGTTGGATGTCATTTCCGCGCAGGATTTG	ACGTTGGATGACGACAGCTTTCGATTGGG	TAGCATATGTGCGCATT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001143	T/C	ACGTTGGATGCACCCAGTTCTGAATACTGC	ACGTTGGATGGATGGCGTCTGACAATGTAG	cTTTATCTAGGTTCTTTCTCTTATATTT
tas0001146	T/C	ACGTTGGATGGTTACAAGCCCACAGAACAG	ACGTTGGATGGATCCAATAAGAATGAAGTG	ATTTTATGTTTGCCATTCTCTC
tas0001149	A/T	ACGTTGGATGGACAAAATGTGGCAACCGTG	ACGTTGGATGCCTCTCCTCGGATTCTTTTC	GCAACCGTGTGATCC
tas0001155	T/C	ACGTTGGATGCAAGGAGATCTGATACACTG	ACGTTGGATGGACCCTTCTATCATTCCCTG	ccacTCCCTGGCAAACCAT
tas0001169	T/G	ACGTTGGATGGACTGTAGCGGAGAGAAATC	ACGTTGGATGACGATTTGCCGCAAATCGTC	cccccCAGACACTTCGAAAATCGT
tas0001172	T/C	ACGTTGGATGGAGTTAGAAAAGGACCAAAC	ACGTTGGATGTGCCAGAGCAACTCAAGTTC	ctcCACTGCAACAACTTCC
tas0001185	G/A	ACGTTGGATGCCTGTAATTGGGAGGTTTAG	ACGTTGGATGCTAAGCGATGTGTTTGCACG	gTCGAGGGTCCAGTTCC
tas0001191	C/T	ACGTTGGATGGTGTTCTCTCTATATCGTGC	ACGTTGGATGCAGGAGGGAAATTGAAAATG	cccccTTCCGTACACAAATTTTGAAA
tas0001208	C/T	ACGTTGGATGGGCTTCATCAATCATGTACAG	ACGTTGGATGTTCGCTCAGCATCATAGCAC	cTGCTGTAAATTGGGCCAG
tas0001212	G/A	ACGTTGGATGACTTCAGTGGGAGCTCATTC	ACGTTGGATGAGGACAGTGCAACGTATGAG	aAGGCTGCTCTAGACTGG
tas0001215	A/G	ACGTTGGATGGGAAAATGTGCAGATAAGAGG	ACGTTGGATGGTCTTATAATTGCAGCCTGG	GTGCAGATAAGAGGAAACTTTTTAAC
tas0001231	C/T	ACGTTGGATGGCAGCAGATATCACAACCAC	ACGTTGGATGACATGTTGCTGTACATGTGG	AACCACATTCTGTGAGAG
tas0001244	C/G	ACGTTGGATGTAGACGAGAATTTAACAAG	ACGTTGGATGGATTCAATTGTCAAGTGACC	agaggCGAGAATTTAACAAGAAATCATA
tas0001248	G/A	ACGTTGGATGTCTGGGAAGATTGAGCATCG	ACGTTGGATGAACCGACAAAAGTCTGCAGG	aCTGCAGAGAAGTCTCTTTC
tas0001252	C/T	ACGTTGGATGCGCAGAAGAAATGCTACTCC	ACGTTGGATGATCTTCCACAGCAAACAGGG	cttcTCCACAGCAAACAGGGATTCAG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001253	G/A	ACGTTGGATGGCTGCATCTGATACGATGTC	ACGTTGGATGGCAGCGTACAGAATACATGG	GGGCGGTGTAATGGG
tas0001256	A/G	ACGTTGGATGGGACAGGGCTGTGAAAGATT	ACGTTGGATGCACTCCGAAGATGCAAAATC	gggaCAGGTTTGTTGACTACTG
tas0001272	A/C	ACGTTGGATGAGTGCTTGCTTTGGCTCCAG	ACGTTGGATGTGAGAGATGTTGCCAGGATG	aGATGAAATAATGGCTAAGTTATG
tas0001274	T/C	ACGTTGGATGAAATGCCAGACCGGTGATTG	ACGTTGGATGCTGCGTATTCAGCTAGTGTG	CGGTGATTGCAATGGACTTC
tas0001279	A/C	ACGTTGGATGGATAAATCATTTACAAGAAAC	ACGTTGGATGGAAATCAAGAGGCTCCATCC	AATCATTTATTTCTAGCTCTCATTG
tas0001289	C/T	ACGTTGGATGTTGATGGGATTGGTGAACCG	ACGTTGGATGCCGCATCACATATGAACAAG	ggggTGGTGAACCGTGGACTT
tas0001298	T/A	ACGTTGGATGTGTTGTTCCGGTGTCAAAGC	ACGTTGGATGTTAACGCAGGCGCAGGAAG	CCGTCGATCAAAGCAA
tas0001306	C/T	ACGTTGGATGCTTGACATTGGTTGCCAAGC	ACGTTGGATGATAACAAACGTTGGAGGTGC	gTCTTGATCCTTTAATGGATAC
tas0001313	C/T	ACGTTGGATGATGGAACTAGCAGGTCCATC	ACGTTGGATGTCAATCTGATCAGTTTCCCC	ggtgAGTAGGATCAGAACCCCT
tas0001320	G/C	ACGTTGGATGGAGATGCTCTAGAGCAATAC	ACGTTGGATGTCATCAAACAGCTTGTCCAG	TCCAGTTCATTACCTTCACAA
tas0001322	C/A	ACGTTGGATGAACAAACTTTCTCCACCCCG	ACGTTGGATGGGACACCACAGTTGTCCTTG	CCCCGCACTTAGACC
tas0001325	T/A	ACGTTGGATGGGACCTTCTGCAATATAACC	ACGTTGGATGGATGGAAGCCGAAGAAAAGG	CCTCATCGACCCATATAGA
tas0001327	C/T	ACGTTGGATGAACCCTGCCTCAGCAATTAC	ACGTTGGATGTGCTTCCAGACCTTTGGAAC	gtttGCAAGTATAAACAAAGCGGAGAA
tas0001328	A/C	ACGTTGGATGCTGAGTACCCAATAGGAGAC	ACGTTGGATGGCCAAAGTCTCTGCAAAATC	GGATGTAAAGAAATTCTGGC
tas0001345	A/T	ACGTTGGATGGGCACTGAATGTGGATCAGA	ACGTTGGATGTGTCCAAGGTTCTATACCTG	agaTTTATTACTGAAGCAACGG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001359	A/C	ACGTTGGATGTCTTCACTTCAAGGAGGAGG	ACGTTGGATGGAGTACCTGTATAGAAAAGAG	ggaacAGAGATCAAGATCTTGACCAAT
tas0001361	T/C	ACGTTGGATGGGAAAGACAGAAGTGGATGG	ACGTTGGATGTGACGATTAGCCTCAATTTC	GATGGAAATGGAGAGTGA
tas0001364	G/C	ACGTTGGATGCCTTACTGTTCATTCAAGAGC	ACGTTGGATGAGACGTTCAAAGGCGTCTTC	ggtAAGAGCAAAAAACATTAATTAGA
tas0001371	G/A	ACGTTGGATGATTGCCAGAACCTAAGCACG	ACGTTGGATGGGCAGAAGAAAGTGGAACTG	TTGGCACCTTCTGTT
tas0001381	T/G	ACGTTGGATGTGCTTTTGCGAGATCGAATG	ACGTTGGATGTCATGCAATCAGAACTTTGG	ggtgTGGTTAATACTCAAACTAGGATA
tas0001394	G/A	ACGTTGGATGCCAATGAGTTACCTGTTGCG	ACGTTGGATGATTGCACCGTGCAGCCAAAG	TTGCGGGAAATCTCC
tas0001399	A/G	ACGTTGGATGCTAAAAGCAGACCTGCTTATC	ACGTTGGATGTGCAATCGAGGTGGATACTG	TCGAGGTGGATACTGTAACTT
tas0001401	C/T	ACGTTGGATGCTACTTAAGAGAAACCAAG	ACGTTGGATGTTGATTTTTCCCCTGCCTGC	TTAATTGTATAATTATTTCATGTGAAGT
tas0001404	C/A	ACGTTGGATGGCCTTTGTATGTGGATGCTG	ACGTTGGATGCGTAAGAGACGTCTTTCGAG	cccacGTGGATGCTGAATCTGTTCT
tas0001407	C/G	ACGTTGGATGGATTTGCTGTAACTATGGTAG	ACGTTGGATGTTTGAGATATTTATTAACAAG	attACAAGAATAAATATTGTTCTCTTT
tas0001412	G/A	ACGTTGGATGTGCATTGAGAAGTGGAGCAG	ACGTTGGATGTTCCACATATTCTACATAG	ccatcGTTTAATCATTCTACTTACGTTG
tas0001421	T/G	ACGTTGGATGCTCATCCTAGTGAAATAAGC	ACGTTGGATGGCACTTGATTGATCATTGCTG	ccctcAGTCAATATTCTGATCCTTTT
tas0001423	G/T	ACGTTGGATGATCAGACCCTGTAAGTTTGC	ACGTTGGATGTTTGTTGTTCTAAGTGCAG	ccctgCTACTTCACTTTTTCAAACG
tas0001427	C/T	ACGTTGGATGGACGAGGCACTCATTAATAG	ACGTTGGATGTCAGGAAGATATGTCGGAAC	GAGGCACTCATTAATAGTGAAATTGA
tas0001442	T/C	ACGTTGGATGTTCCTCAACATCTCCACTGC	ACGTTGGATGGAAAGTGAGGAATCTGGTGC	GCTCACACCCTTCTCC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001444	G/A	ACGTTGGATGCGATATGAGGAGTTGTCTCG	ACGTTGGATGACGACATTTGAAACTCCCCC	ccTGGAGTACAAGCCTCA
tas0001450	A/G	ACGTTGGATGGAACATATTTCAGCCACGG	ACGTTGGATGGAAGTGCTGTGTAGTTCCTC	acCTAGTGAAGTCTAATTTCAGGAG
tas0001457	C/T	ACGTTGGATGGTGATTGTCAATCCCTAGAG	ACGTTGGATGTTTCCACAGTTGGAAATAC	tGAGTTCTTTCCTATCAGTGGT
tas0001464	A/C	ACGTTGGATGCTTATTTGTCATGAATCCAGC	ACGTTGGATGCTTGTAAGGTGTGGATAAGG	TGTGGATAAGGCCTTTC
tas0001470	C/T	ACGTTGGATGACAGCTATGTCACTTACGGG	ACGTTGGATGACTGATAGACCAGTCAACAG	ccgagAGACCAGTCAACAGACCCCTTC
tas0001480	A/T	ACGTTGGATGCCAATACTTGATCTCTAATTC	ACGTTGGATGTTCTGTGCGCTTGTTACTTG	cctcgCTAATTCTTGATTTCAAACCGA
tas0001487	C/G	ACGTTGGATGGGCGAAATATTGACTCCATC	ACGTTGGATGCCAGTTTTTTTCTCCCCTAC	tttgTTCTCCCCTACATTAGTTAAAACA
tas0001493	T/C	ACGTTGGATGGATCTTTCAGTACCTCTCGG	ACGTTGGATGCCTTGCTTAACATCAACGCC	ggaTCTCGGTTTTCGGGC
tas0001495	G/T	ACGTTGGATGGGACCATTCTGCATCCAGTT	ACGTTGGATGGAAGTACTACAAATAAAACG	ATTCTTTTACAAAATCAAAATTATCA
tas0001499	C/T	ACGTTGGATGATGACTCCCAAGATCGTCTG	ACGTTGGATGCGCGAATTTGAATGCAAAAC	GTGCCATTACTCATAAACTC
tas0001523	T/C	ACGTTGGATGGCTTGCAAAGCTGCAAGTTC	ACGTTGGATGGAACGTTCCAGCTGGTAGAG	ggGCAAGTTCATCTTCCTCCTC
tas0001527	T/C	ACGTTGGATGGGTCTAGGTATTCCTTTCGG	ACGTTGGATGAAAAGCAATGGATTTCTTC	ccctATGTATGTGTGTCCCC
tas0001533	G/A	ACGTTGGATGGGTGCGAATGAAAGTCATCC	ACGTTGGATGGGTGAAATCACTGTGGTTGC	AGAAGAATAAATATTCTGAACTTG
tas0001535	G/A	ACGTTGGATGCCACTGAGTTGCATTTCTCC	ACGTTGGATGCCTGGTGGAATAAACCAAGG	aatAAGGGGTTGACGGTG
tas0001538	T/A	ACGTTGGATGATCGCCAAACCTTCAAAAGC	ACGTTGGATGCCGTTTTTCATTTTATTCTG	CCTTCAAAAGCACCAAA

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001543	G/A	ACGTTGGATGGAGTTGGGGTAGTGTTTATG	ACGTTGGATGCTCAGAATCATATGTAGCAAG	gATGTATACTCTTGGTATTAGAAGT
tas0001544	G/C	ACGTTGGATGTAAGCTCACCCAATCACCTC	ACGTTGGATGAACTCCACGACCCATGTCTC	AGCCTGATCGCCTTT
tas0001545	C/T	ACGTTGGATGAGGATCCGGATGATCTGTTC	ACGTTGGATGCTAGCCCTAAAAGAGATGAG	GAAGAAAGAGAAGAAGGC
tas0001562	A/G	ACGTTGGATGGCCAAAATCCAAAGGCTCAG	ACGTTGGATGCAGTAAGAAGAGACGAAGGC	attGGGAGTCGAGCAGGG
tas0001564	C/G	ACGTTGGATGTTAGAGCTGATGAAGAGGCG	ACGTTGGATGTAAATAAGCTTAGCTAGAC	CGGTCTACCTACCAACT
tas0001568	T/C	ACGTTGGATGGTAGCATCAATATGCACCCC	ACGTTGGATGCGAAAAACTGTAACTGGCCC	ccaaCCCTTGTCACAGTCCTTAGTA
tas0001579	C/T	ACGTTGGATGTTGCAGCTCTGTACAAACTC	ACGTTGGATGAGGAATTACCAAGTTCCGTC	tttctTTCCGTCCACTTTGGACCA
tas0001585	A/T	ACGTTGGATGGCACGGATTCCTTAATAGGG	ACGTTGGATGTTCTTTATAGCTGCAGCCCG	GGCTCATATGGAAGAGTATATT
tas0001590	T/G	ACGTTGGATGCATGAACAAGACATGTACAC	ACGTTGGATGCACATGAAACACTTGTGTGG	AGACATGTACACAAAGCTCAC
tas0001595	C/T	ACGTTGGATGTTAGAAGGGTGCCCCATTTC	ACGTTGGATGGCTTGTCTATGGCATTCAGG	cCAGGACCCAGCCAAC
tas0001603	G/A	ACGTTGGATGGGGAACTTTTGGGTAAGTGG	ACGTTGGATGTACAGTTCCTGGAGGCTTTC	agttGTTGTTTAACAGTAATGTTCAC
tas0001604	A/G	ACGTTGGATGATGTGTGTGCAATACGTAGC	ACGTTGGATGCATGTGAATGTTACTCCACC	ccccGCAATACGTAGCGGTTGTCC
tas0001607	T/C	ACGTTGGATGGCAGCCTGAATAGAAATGAG	ACGTTGGATGGATGTCCGACAGGTTCACAA	caCAGATATGCTCTAGTACCAT
tas0001612	T/C	ACGTTGGATGTCCAGAATGAATCAGAGTTC	ACGTTGGATGCTATTTACTACTGTTGGGGC	AAAGAATATGTAAAATGCGATGAC
tas0001614	A/T	ACGTTGGATGAGGAGCAAGCACGTAGGAAG	ACGTTGGATGGAGCCTTACAAACCTCCATC	cccctATTCCATCTTGTGCACG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001620	T/A	ACGTTGGATGCCAAGATCTTTTTCCTTCGC	ACGTTGGATGTCCGCTGTTCTAGATCTAGT	agataTTTCAGTTTGTCTCAATTCT
tas0001622	G/A	ACGTTGGATGCATGCCAAATCCAATGCATC	ACGTTGGATGAGACTGAGAATGAACTTGGG	gggttAGAATGAACTTGGGATTTTC
tas0001631	T/G	ACGTTGGATGAACCCCTCAACTTCTTCTTC	ACGTTGGATGGGTGATACTGTGTGTAGGAC	TCAACTTCTTCTTCTACACAA
tas0001636	T/C	ACGTTGGATGCAGACAAGAAAACCGCAGAC	ACGTTGGATGTTTGTTCCTGTGGATGAGCG	ccCAGACCCACCAACTT
tas0001639	G/A	ACGTTGGATGCCAGAAGCCCTCTTGACAAC	ACGTTGGATGTGGTTACGACTCTAAGTCTG	ggCTGGTCGACCCTAAAAC
tas0001645	A/G	ACGTTGGATGTCGTAGCTTCTGATTGTTCC	ACGTTGGATGGCTAGTCTGTTCTTGCTTTC	ccccGTCTCTTTTCTGCAACATATCTGTC
tas0001648	T/C	ACGTTGGATGGGTACTATTGACTACCACTC	ACGTTGGATGGCACCACAACTAAGGATCTC	tgacgACCACTCATCTGCCGTTAC
tas0001653	G/A	ACGTTGGATGTTGTTGTGCAGTTACACTCC	ACGTTGGATGTTAGCAAATCGAAGTACCCC	cTGCAGTTACACTCCCATATC
tas0001655	A/G	ACGTTGGATGAACCCTTTCTTCTTGCTGCC	ACGTTGGATGTTTAGGGAGGGCCATGATTC	cTACAGCAGCGGTAGCCC
tas0001662	C/T	ACGTTGGATGCTTGCCTGTGATTGGTGTTC	ACGTTGGATGCAAGTACCTGAACGATAGAC	GCGTGCATCTACGTTAGA
tas0001669	T/G	ACGTTGGATGATGCAGTCATTTGGTTCTTC	ACGTTGGATGCTGAATATCCTAGTACCAAAG	CCAAGTCAACGCAATATG
tas0001672	C/T	ACGTTGGATGGTCTATGATGGTTGTTGGAG	ACGTTGGATGATGTTGCCATTGCTCCATGC	GTTGGAGAGAGGGAGTA
tas0001689	G/A	ACGTTGGATGGAATTCACCACGATCCATGC	ACGTTGGATGACCTTAACCCAGGGAAAAGC	ggtCCTGAGAAAACTTTTGCC
tas0001690	G/A	ACGTTGGATGTGTGATGCATCATCTTCAGG	ACGTTGGATGTAGAAGCACAACCCCAAAGC	GCTATAGACATCACTTTTCTC
tas0001706	T/A	ACGTTGGATGCTGAGGTTGGTTGTCTTAAA	ACGTTGGATGCGCAGTTCCAAAACCTCTTG	gGCAAATTCTAATGATACTTTTAGATTT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001716	G/C	ACGTTGGATGCTAGTGAAGGTGACACAAAC	ACGTTGGATGTAATTGTCCACATCCCCCAC	ggggACCTTTGGGTATGGTAATTT
tas0001717	G/A	ACGTTGGATGTTATCACTAGTAAGCGCTCC	ACGTTGGATGGTGGCAGCATAAATTAATGTG	caATAACTATTTTGTGACTACCATA
tas0001719	C/T	ACGTTGGATGCACTCTCGCCATGTTATCTC	ACGTTGGATGAATCCGTGCAATGCTGAATGG	ctgaTGATGGAAACTCTCTAGTTAT
tas0001733	C/A/T	ACGTTGGATGCATTCAATCAGCACCAGAGG	ACGTTGGATGGAAGTAAGAAGCAAATCGTTG	tccaCAGAGGAAAGCAGATCA
tas0001740	C/T	ACGTTGGATGTCCATTCATTCTTCCGGAAC	ACGTTGGATGATTTTCTCATTTACTGCTC	aTCTGTTTTTTGAACCTTACATA
tas0001746	A/G	ACGTTGGATGCAACAAGAAATATCAATGGTC	ACGTTGGATGACTGGGATTTTGTTTGATG	ccacACTTAAGACAACTTATGCG
tas0001750	G/A	ACGTTGGATGGTGCTTATCCTGTAGATGGG	ACGTTGGATGGGTGCCATTTCTTTAGAGCC	cccatCCTTTGCGGTTTGTTC
tas0001760	G/C	ACGTTGGATGCTTGTTGAACTAATGAATCC	ACGTTGGATGACATCAGATTGGTTAGAGTC	gGAACTAATGAATCCCGAAGG
tas0001766	G/A	ACGTTGGATGCTGTGAGAAACTCGAACGTG	ACGTTGGATGTTTTGTCAGACATCCCGGTG	gGTTTTAACTTCCCTTGACC
tas0001767	A/T	ACGTTGGATGACAGGGCAATGTTAGAAGGC	ACGTTGGATGTCCAAACTCAATCGCCTACC	CCAACCTTCCCATTTCC
tas0001769	A/C	ACGTTGGATGTTACACTTCCAGAAAAAGGG	ACGTTGGATGTGAGGATCTGCCTTATCTTA	ggggAACTATTAGGCATTATTGAAGAC
tas0001773	A/C	ACGTTGGATGTGCCCTCTTGTTTTACCGAC	ACGTTGGATGCTTTAGTCCAAACAGAAGGG	aTCTTGTTTTACCGACTCAATATATTT
tas0001777	G/C	ACGTTGGATGCAGAATGGAGGAGGCTTATG	ACGTTGGATGTGGATCTTGCAGCCATTCAG	gtAAATCACGGATTCGACTATTG
tas0001785	A/G	ACGTTGGATGTTCTCGCATGGCGAGTTTAG	ACGTTGGATGTTTAGCCAAGTAGCTCCGTG	cccccTTTGAAAAATCCTACCGTGA
tas0001790	A/C	ACGTTGGATGGTAAGAGGCCTGGTAAAGAG	ACGTTGGATGTATTCAGCACCATCTCCCAC	CTGCCATTTCCACTAATT

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001796	C/T	ACGTTGGATGCAATCAACAATCGAAGGCGG	ACGTTGGATGTTGGGATTTTGCTTTTGGAG	gGCTTTTGGAGATCATTAGTGG
tas0001800	T/A	ACGTTGGATGACTGGATTTAAAGTTGGTGC	ACGTTGGATGTTTCCATCACTGCTAAAAG	AAGTGAATTGGACCAGA
tas0001801	T/C	ACGTTGGATGCTCCTCTTAGGAACAGTACG	ACGTTGGATGAGAGGCGGCGGATGAATTAA	aCCCGACTCAACTGAATGTATAG
tas0001810	C/G	ACGTTGGATGTGAATCCCCACCTCAATGTC	ACGTTGGATGAGGATCCGAATTGTCCAGAG	cTTGTCCAGAGAAACTGTTTATTT
tas0001816	C/A	ACGTTGGATGTATAAGAGATGGAGATGAGC	ACGTTGGATGCCTGGTGTAAGCTTCTATGG	gATGGCTGTTGTTTCTAAATT
tas0001827	T/C	ACGTTGGATGAAAAGTTCTGCTGGGGCATC	ACGTTGGATGATTCTGCCCTTGACCAAACC	cccaAGCATTTGTGGGGAC
tas0001832	G/A	ACGTTGGATGGCACTCAACATACCTGGATG	ACGTTGGATGTTCCTTCCTAAGAACACACG	tttcTCCTAAGAACACACGTTTTCAA
tas0001834	A/G	ACGTTGGATGTGATACACTTCTAAGCAAGG	ACGTTGGATGGGTTCGCAAGAGGAATGCAG	cgtcATGCAGCTTTGTAATTAGATT
tas0001835	G/A	ACGTTGGATGAGTGTTACAGCTTGATGTTC	ACGTTGGATGCCTCTCATTGTAATTTAGG	tttttAACTAGATGCTGCCTTTT
tas0001847	T/C	ACGTTGGATGGACATTACATTTCTCTTAGC	ACGTTGGATGGGCAAAAGCTCTTGAAGAGA	ggagATGCGTGATCTTCGTGCCCAGGA
tas0001851	T/C	ACGTTGGATGTCCGGTCAGAGTGCTAGTAA	ACGTTGGATGACCATTGTACCCGAAAGCAC	tttgaACGGTAATAAATCCACAGAGAAC
tas0001857	A/G	ACGTTGGATGTGAGAAATATATCTTCCTC	ACGTTGGATGCACCATGACTACCTTAGAAG	gtACTACCTTAGAAGAAATCAATCACTG
tas0001862	C/T	ACGTTGGATGGTTCACGATATTTCAGCTCC	ACGTTGGATGTATAGAAAGTTTGTTCTGG	TCCATTTTACACATATACATCAAGT
tas0001871	G/C	ACGTTGGATGACACAAGTTTGCAAGTGAAG	ACGTTGGATGGTTGGAGAAGATCTTTATCG	ctAGAAGATCTTTATCGATGATTG
tas0001878	C/A	ACGTTGGATGCCAATGCAAGCACAACCAAA	ACGTTGGATGATGTATCTCTCCCAACTTGC	tcccTTGCAGCTTAAGATTCTTGTG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tas0001889	A/T	ACGTTGGATGGGTAGCCTTTGTTGAAAGAG	ACGTTGGATGAGCCTCTCAATGTACTCACG	ctccATGTACTCACGCGTATG
tas0001906	C/T	ACGTTGGATGGCTTCAGTCAACAAAAATA	ACGTTGGATGTGCCTCCTATTTCCCAACTC	ggaaCAACTCAAAGATTTAGGTTGC
tas0001913	T/A	ACGTTGGATGATGGTCTGTCATTTGTCACG	ACGTTGGATGGACAACACAAGTGGGACAAG	agGTCATTTGTCACGCTAATA
tas0001921	G/A	ACGTTGGATGTTATCTGGTGTTGCAGGAAG	ACGTTGGATGAATAATCCGCAATGCCTCCC	ccCACATCCAACCTCCC
tas0001924	C/G	ACGTTGGATGACCAGCATCAAAGTAATCAC	ACGTTGGATGCCTCATTTCAACTCATGGTG	ATGTGATTGTAATTTAAATTGCATGATA
tas0001937	C/T	ACGTTGGATGCCCGATGTACTTGCAGTTTG	ACGTTGGATGCTGGAACTTCTGATGTATCG	TGTATAAGACCCAGAGGA
tas0001943	C/T	ACGTTGGATGGGAACAAGATACTGGCAGAG	ACGTTGGATGTGTGCAGTTGACGTCCTCTC	tctGTCCTCTCCCCCTCT
tas0001945	T/C	ACGTTGGATGCGGGCTAATGAAAGATCAAA	ACGTTGGATGTTGAAGATGCTGCAACTCAC	agacGCAACTCACATCATGAATC
tas0001954	A/G	ACGTTGGATGACAGGCTTTTCCAGAAAAGG	ACGTTGGATGAATATGCATGGGCCGACAAG	ggagTTTCCAGAAAAGGAAATTGTTAT
tas0001966	C/G	ACGTTGGATGGCTAACTTTGGTTTCTTTCC	ACGTTGGATGGGCATTTACTTTTTTTCGGC	cTTTTTCGGCTGATTCCTC
tasCp20_12116	TTT/AAA	ACGTTGGATGAACTCTCGGGAGAATCGATG	ACGTTGGATGGGGCGATTTACCCAATTACG	TCTACTAGCACTGCATC
tasCp20_16837	C/A	ACGTTGGATGGGCGAAATACAGTTCATCAG	ACGTTGGATGGGTCTTTTGAATGACATTTGC	cctccTCAGCGTGGTATAGCTGAAAATG
tasCp229_16879	C/G	ACGTTGGATGGCACTAATCCTTTCAATCCG	ACGTTGGATGTCGATTGGTTGAAAGGCCTG	gggcCTAATCCTTTCAATCCGGTACCAA
tasCp229_2616	T/C	ACGTTGGATGCAGCGAAAAATGCCATTGCC	ACGTTGGATGTTCTGGCTTCAAAAGGGACG	cATGGAAATGTTACCTTGTC
tasCp229_3114	C/A	ACGTTGGATGTTCCACATGTATAGGGTGGG	ACGTTGGATGATTTCCCTTTTTAGAGGAC	CCTATATCTAAGACAAAATTTAAATGTG

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tasCp229_8690	G/A	ACGTTGGATGGGCCCCCTTTTTTTTGTTCC	ACGTTGGATGGTTTTCAAATCAGATATCAG	agTTCAAATCAGATATCAGATAAATCAT
tasCp373_606	DEL/C	ACGTTGGATGCAGAATTAGGGATTCGTTGG	ACGTTGGATGTCATCCTATTGGTTCGGCTC	GTTCGGCTCATACGAAAAAAG
tasCp373_68	G/C	ACGTTGGATGCAAATAAAGCCCGAGGATTG	ACGTTGGATGCATCATAGGCACATTGGGAG	CCCGAGGATTGGCATTC
tasCp436_10090	G/T	ACGTTGGATGAGTGCTTCTGAATTCATCTC	ACGTTGGATGCAAGGCTAAAGTTATTAATC	gggAATCAACAAAGAAAAATGAAAATT
tasCp436_10452	DEL/C	ACGTTGGATGTACTCTGGATCGAAATCGCC	ACGTTGGATGAAAGAAATTCGAATTGGGGC	aggCGAATTGGGGCTTTAAGTTGG
tasCp436_2600	G/C	ACGTTGGATGTTAGGCATATTTTTCACCC	ACGTTGGATGGTCTGCGCTTTATTCATTATG	GCATATTTTTCACCCATTTTAT
tasCp436_3740	A/G	ACGTTGGATGCATCAATTTGAATCTTTCC	ACGTTGGATGCGGCTTGTCTTGAACTCATA	gTGAATCTTTCCTAAAAAAATATTACC
tasCp544_15768	T/A	ACGTTGGATGGAGTTAGAGCTGTAGCTATG	ACGTTGGATGGCTCCCGTATCAATCACTTC	tcccTCCTCTCGTCAGACC
tasCp544_19217	A/DEL	ACGTTGGATGGGAAATACCTATCCATTCTC	ACGTTGGATGATCAGGAGGTCTTTTTGTCG	TCGTTTTTTCTTAGTGATTTAGA
tasCp544_19813	TTT/CAA	ACGTTGGATGCAAAATCATGACACGAGCCC	ACGTTGGATGCTTGTCCACAAAAACATCAGG	TCCCTAGATCTATGTGAAAAACTCT
tasCp544_3958	A/T	ACGTTGGATGTTATTCTTTCTTCGCTTTC	ACGTTGGATGTGGAATCCCCCCTTTTTTTC	TCCCGAATAGTAGATCCTA
tasCp544_750	DEL/GGGA	ACGTTGGATGTTAAATGGAGTTCCCCGGAG	ACGTTGGATGCGTGTTCATTCATTTTGAC	TCATAGTAATTTCGACTCCC
tasCp612_1890	C/T	ACGTTGGATGTAGAAACTGCGGCCGTTAAG	ACGTTGGATGGGTTCACAGGTACAACCTTT	cccccCAGCCTTCCAAATAGGAACTGGC
tasCp784_4541	A/G	ACGTTGGATGAAAGCGCCCGTAATAAAGAC	ACGTTGGATGGGTTCGAGAGAAAGTAAGAG	CGAGAGAAAGTAAGAGTATCTA
tasCpIN373_7345	DEL/AATAC	ACGTTGGATGATTTTCTTAAAAGAAATTTCG	ACGTTGGATGAGATAGTTAATTTAGGTTTC	TTTCGAAATTTCAATTAAAACTAATAC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tasCpIN544_750	DEL/GGGA	ACGTTGGATGTTAAATGGAGTTCCCCGGAG	ACGTTGGATGCGTGTTCATTCATTTTGAC	CTCATAGTAATTTCGACTCCC
tasMt12709_853	T/G	ACGTTGGATGGCTGCAAGAGGTTACTAATC	ACGTTGGATGGCACTCCTTAAACCGCTTTC	TGGAGGTGAGGGAAGA
tasMt1274_1310	T/C	ACGTTGGATGTAGGAGTAGAATGTCAGAGG	ACGTTGGATGCTTGTCTGCTTCCTCCAAAG	TTCTAGGGTCACTCTCT
tasMt1438_1478	C/T	ACGTTGGATGGGATGTTAACGAAGGTGAGG	ACGTTGGATGTCAGATACGTTCGTTCGGGTC	GGTGAGGGCCTCTCC
tasMt1438_2665	T/C	ACGTTGGATGTCGAAGACCTCCTTACGAAG	ACGTTGGATGATAACGGAACTGCCTGAACC	TGAACCGGCGTATGG
tasMt1485_2465	A/DEL	ACGTTGGATGGATAGTCTGACCAAAGGAGC	ACGTTGGATGTTTTCTAGGGTTGGTGTGGC	GGGCCTCAGACTGAAT
tasMt1580_3099	A/C	ACGTTGGATGTTACACCTTCGGAATACCCC	ACGTTGGATGTATGACGAAAGTGGAGTCGG	cccgAAGTGAAGATTGGGGGAGTAGA
tasMt1580_4320	T/A	ACGTTGGATGTGGGTGGGCAACTTCTATTC	ACGTTGGATGAGGCACATTGTTGTAAGGCG	ggagCTGGGAACTCTGAAGTAC
tasMt1580_5870	A/T	ACGTTGGATGGTTTGGCTCAACGCCAATAG	ACGTTGGATGCCTTTTTCCAGTCCCTTTTG	cccTTAAATAAATTGCGACAACAC
tasMt1718_3790	A/G	ACGTTGGATGTTTGGCTGACCCTCTTCTTC	ACGTTGGATGACTCTCCAGTGGTTCCATTC	gtgggTGGGTTGACCCAGAAGTACA
tasMt173_2622	C/A	ACGTTGGATGCAAACCGTAGCAAGCCAAAG	ACGTTGGATGCCACTCGTTTTGAGTTCTCG	ggggcGTTCTCGATGAGCAAAAGATT
tasMt173_3690	DEL/TCTAT	ACGTTGGATGGATGAAAATAGAGGGATTGGC	ACGTTGGATGTGGCCTACTCCTTACTCAAG	GCAGTATGCAACCGC
tasMt1751_2224	G/T	ACGTTGGATGACACCCTCGGTACACTATTG	ACGTTGGATGTCTGTGAAAGATGGGATCAG	agCTATTGAAGATCTCACAAAGA
tasMt2011_567	G/T	ACGTTGGATGAACCCTCCCTACCAGCTTCA	ACGTTGGATGAACCGGTGCTAGTCTTTGAG	TACCGCTGCACAAGT
tasMt2580_2831	A/C	ACGTTGGATGCATCAGGAACGAAGTCACTC	ACGTTGGATGTCCTACACCGGTGTTTGATG	GTTTGATGCGATGGGA

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tasMt261_3241	CTTT/AAAG	ACGTTGGATGTGAGAACTCTTGTTGGCAGG	ACGTTGGATGTTCTTCCCCTCAGGTCAAAG	aaaaTCAGGTCAAAGAGTAAGAACTCG
tasMt290_8875	G/C	ACGTTGGATGTTCTCACTCCCCTTCAACAC	ACGTTGGATGCGAGGAATTCTAGTCCAGAG	ccccgCGACTTCATTCTTGTAGTCATG
tasMt316_2642	TC/GA	ACGTTGGATGAATGGTAAGCCGGGTCAATC	ACGTTGGATGGCCGCATCCATCGACTTTTA	cGCTTTCTTAACTTCCGAATT
tasMt319_5699	A/C	ACGTTGGATGGTTCCATTTAGTCAAGGGAG	ACGTTGGATGACGAACTAAGGCGTGCTTTG	gtTCTACCGATGGGTTGAT
tasMt3293_614	G/A	ACGTTGGATGCCTCCGACCATAAAAGAAGG	ACGTTGGATGTGATGCTTGCTTGATGGGAG	ATGGGTTGGGATCCTGC
tasMt363_11263	C/A	ACGTTGGATGGAGGGCGTCCTTTTCTTCTG	ACGTTGGATGATGGCTAATGTCCGGTACTG	gatGTACTGAGCCGATAGC
tasMt363_15481	TTA/DEL	ACGTTGGATGGAGCTATGAAGCTTACCAAC	ACGTTGGATGAAAACGTAAGTGCGCTCCTG	ccccAGCCCTTTCCCTTTTTAATAA
tasMt363_3718	G/T	ACGTTGGATGGCAACTAGTTCTTCCATGGG	ACGTTGGATGTTGTCTTGGGATACGAGCTG	CTTCTTTCAGAGAAATCAAGTATTAT
tasMt363_9461	G/T	ACGTTGGATGATCATTCTATTTGTTCCTC	ACGTTGGATGGAAGGTTAGACAAAGACAGC	gcTCCTATATAGGATAGGGGAATT
tasMt4446_1048	A/T	ACGTTGGATGCGGTAGTGAATCACCTACTC	ACGTTGGATGAACATGAAAGAGTCCACCGC	CCTGCCTTAGTAGTCTC
tasMt4446_149	G/T	ACGTTGGATGTCTCGGTTGTAGTCTGATTG	ACGTTGGATGGATCGATTAGCTTAGAAGGG	CCTCCCATTGCTAGG
tasMt4446_1728	C/T	ACGTTGGATGCTCTCTTTCAAACAGTAGAC	ACGTTGGATGGAGTTATGGCATTCAATCTC	cccccTTTTGCTTGTGCTAGTG
tasMt4446_2766	T/C	ACGTTGGATGCTGAGATGTTCAGTCGACTC	ACGTTGGATGCTCTTTCCTCCTCCCTTTTC	CCCTTTTCTGCTCACT
tasMt4446_563	T/A	ACGTTGGATGAATAGATCAGATCCGGACGC	ACGTTGGATGATTCCCGACATGCTATGGTG	aggaaGTAGCGCCGCGGAGC
tasMt4638_237	G/DEL	ACGTTGGATGGACGTTGAACGCTTCAACTC	ACGTTGGATGGGCAGTAAGCAGTAAGTTGG	gtCCTAGTCTCTCGCCC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(continuation)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tasMt52_15844	DEL/T	ACGTTGGATGACTTCCTTCATCATGGATGG	ACGTTGGATGTGGTTAGATCGCCTGACTTG	ctgtAGCCTTTCATTGCTTATTAGAAAA
tasMt52_22901	GAC/CTA	ACGTTGGATGACTTAGGATGGAGTCCTTGC	ACGTTGGATGCCCGGAAGAAAAAGAAGAGG	CGAGCTGGAAGCTATACT
tasMt528_151	A/C	ACGTTGGATGCCTTAACTAAACGGGCATTC	ACGTTGGATGCGGAGACGGTAACATTTTCT	cCGCTTTTTACCTTTCATTTTC
tasMt53_18528	TC/GA	ACGTTGGATGTGAAGGCTAAGGTACCTTCC	ACGTTGGATGCCTTGAAGCCAAAAAGGCTG	ACCTTCCTAAACTGAGATT
tasMt53_8442	DEL/CCTG	ACGTTGGATGAGTTCCCGTTCAGTGATTGC	ACGTTGGATGTGGGCTCCTTCTCCTTTGC	catagCTCCTCAACCCTCATCCTG
tasMt6079_1485	A/C	ACGTTGGATGTACTATTCCCTGTCTTCGGC	ACGTTGGATGCGGGACCTATGATTGGTTAC	ttcaTGGAAGAAAAAGGATAGATTT
tasMt615_3863	A/C	ACGTTGGATGAGAAAAGTGACCGACCAGAG	ACGTTGGATGATGAATCGGGATCCGTATAG	cgCCGTATAGTATAAAGGAGGTTT
tasMt615_5988	T/G	ACGTTGGATGAGATTGATCAAGCGACACCC	ACGTTGGATGGTAACCTTTTTGCGGCATGG	ggAAGGCAGGGGACTTA
tasMt680_10665	T/A	ACGTTGGATGATGCCTGGTCAACAACCAAC	ACGTTGGATGGACAGAAAGAGAGTCCTTCC	tcaGAGTAGTGAAGAAGGATAGT
tasMt680_8937	A/G	ACGTTGGATGATCTTCGACTGCATCGCATC	ACGTTGGATGAACTGGACGAACGCATCAAG	CCCCTTTCCTGAGCG
tasMt732_3426	G/C	ACGTTGGATGGAGAAATACTGTCAAGCGGG	ACGTTGGATGAAAGTCTCTGCGGAGCCATC	GGAGCCATCTTTGGT
tasMt735_8582	T/G	ACGTTGGATGGGTTTCCTTTGCTTTGGTCG	ACGTTGGATGAGCTCCACTGATTCACCTTG	TGGCCGATCTAGCAAGATT
tasMt735_9652	A/T	ACGTTGGATGAGAACAGTTCGAACACAGGC	ACGTTGGATGCCTTCCCTACTACTATCAAC	gggaACAGGCTAATATAATCAACAAGAA
tasMt781_6951	TC/GA	ACGTTGGATGCTGCAGAGGTAAGATCCAAG	ACGTTGGATGCACTTGAAAGGAAGAGCAGG	ccGGCTAACCTAGTATCCCTCT
tasMt79_13859	GTT/TAA	ACGTTGGATGTGTATCGCCATGCTATTAAG	ACGTTGGATGACGCTTCAACCGGGTTATTC	ctagTCCACCTCTTAGAAAGAAAAGAAC

Table 2- Set of 402 markers. Details of each marker type (SNP/INDEL) alleles, forward primer sequences, reverse primer sequences and extended primer sequences.

(conclusion)

Marker name	Allele	Forward Primer Sequence	Reverse Primer Sequence	Extended Primer Sequence
tasMt79_14230	C/G	ACGTTGGATGGAATGCTAGTTCTTACTGTTC	ACGTTGGATGGCTTTAAGTTCTGGAGGACG	gtACGAAATTTCATCATCCATACATAAC
tasMt79_1805	A/G	ACGTTGGATGTTCTCCTACACACCTTTGCC	ACGTTGGATGATGGAAATTTAGGTCTCTGC	ggaTCTGCAAGATTATTCTTTCC
tasMt79_21228	G/A	ACGTTGGATGGCTGCTTACTTTGGTGCATC	ACGTTGGATGAGGGACTTAGATGGAGAACG	gtAGAACGAAGAGAAGAAATAAG
tasMt8925_465	T/A	ACGTTGGATGAGGAAAGCGCCCTCTTTTTG	ACGTTGGATGGGAATCTTGTAAAGGGCAGG	TGGCCTATTGGGATAGC
tasMt965_6474	DEL/TTAG	ACGTTGGATGTGCCCTCCAGCTCAGATTTC	ACGTTGGATGGTGTCTGAGCTGAGAGAATG	TGAGTTCCCCCCCAA
tasMt967_4264	G/T	ACGTTGGATGAGCTGTGAGGCTTAAGAGAC	ACGTTGGATGGTCGATTCATTAGGGTCGTC	aAAAGAAAACGGTGGATACTTT
tasMt967_4329	C/A	ACGTTGGATGACGACCCTAATGAATCGAC	ACGTTGGATGTGAGATTGCCTGCTTGCTTC	ccaaTGTTAGCTACATCAGCACT
tasMtIN1006_5914	DEL/ACAA	ACGTTGGATGTATCCTTTGCTGGCTGGTTG	ACGTTGGATGTTTGAGACGAATTCATTCCG	CAAGGAGTAGTCCTTGT
tasMtIN173_1921	DEL/AGCGA	ACGTTGGATGGATTTCAGGCGAGACCATTG	ACGTTGGATGGATCAATGGTAAGGCATCAC	TCTGGGTAGAGGAGC
tasMtIN227_7763	ACTTA/DEL	ACGTTGGATGATGCTCGTGCATACAAGGAC	ACGTTGGATGGGCTAATAGTAAGGAGTTGG	GTTGGAGAAATCAAGTAAGT
tasMtIN2369_2284	CCAGG/DEL	ACGTTGGATGCTGGCGCAGGTTTTTATTCC	ACGTTGGATGCTGTTGACTCTACCAGATAG	tTTCCTTCTGAGCCAGG
tasMtIN857_1034	GGGTC/DEL	ACGTTGGATGCCTTATCTGACTGAAGAAGA	ACGTTGGATGCCATACTTTCTCTTTAGAGC	gaggTAGAGCTCAATTACATCGACCC

Table 3- Location, species, number of individuals and geographic coordinates of individuals used for the initial SNP selection on the MassARRAY® iPLEX™ platform (Agena Bioscience™).

Land	Location	Species	Number of individuals	Latitude	Longitude
Bolivia	Bajo Paraguá	*H. serratifolius*	5	-16.0907	-61.1490
Bolivia	Concepción	*H. serratifolius*	5	-16.1674	-61.7679
Bolivia	Guarayos	*H. serratifolius*	5	-15.5148	-63.1971
Bolivia	Roboré	*H. serratifolius*	5	-18.4272	-59.4516
Bolivia	Rurrenabaque, El Paraiso	*H. serratifolius*	1	-14.5667	-67.3033
Bolivia	Yapacaní, Naranjal	*H. serratifolius*	7	-17.4293	-63.9151
Brazil	FLONA do Jamari	*H. incanus*	5	-9.3965	-62.9226
Brazil	FLONA do Jamari	*H. impetiginosus*	5	-9.3985	-62.9248
Brazil	FLONA Amapá	*Handroanthus* sp.	6	0.8668	-51.6012
Brazil	FLONA de Tapajós	*H. serratifolius*	8	-2.8641	-54.9269
Brazil	FLONA do Carajás	*Handroanthus* sp.	13	-6.1971	-49.0848
Brazil	RESEX Chico Mendes	*Handroanthus* sp.	3	-10.7751	-69.6563
Brazil	RESEX Tapajós - Arapins	*Handroanthus* sp.	5	-3.0783	-55.2841
Brazil	Piracicaba, Brazil	*H. rosea albus*	1	-22.725	-47.6476
Brazil	Piracicaba, Brazil	*H. ochraceus*	1	-22.725	-47.6476
Brazil	Piracicaba, Brazil	*H. heptaphyllus*	1	-22.725	-47.6476
Brazil	Piracicaba, Brazil	*H. chrysotrichus*	1	-22.725	-47.6476
Brazil	Piracicaba, Brazil	*H. impetiginosus*	1	-22.725	-47.6476
French Guyana	Belizon	*H. serratifolius*	5	4.2089	-52.5480
French Guyana	Paracou	*H. serratifolius*	2	5.2601	-52.9361
French Guyana	Kourou-Cayenne	*H. serratifolius*	8	5.0020	-52.4808
Germany	Bergadorf	*Tabebuia barbarata*	1		

Source: author of this study.

4.3 RESULTS

A total of 402 SNPs/INDELs markers were identified for the genus *Handroanthus* sp., and being able to have primers designed to be multiplexed. The overall amplification rate was of 86.74%. Plastidial markers (77 markers) presented overall 92.22% of amplification rate, while nuclear markers (325 markers) obtained a success of 85.49%. The mean $\delta_{Gregorius}$ (GREGORIUS; DEGEN; KOENIG, 2007) among nuclear markers was 0.192, among chloroplast markers was 0.405 and among mitochondrial markers was 0.399. Further

information on genetic diversity and differentiation will be presented in the Chapter 3 of this dissertation.

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5 CHAPTER 3: DNA FINGERPRINTING OF *Handroanthus* sp. USING SNPs MARKERS

ABSTRACT

The genus *Handroanthus,* known “Ipe” or “Lapacho”, presents one of the most valuable timbers from South America and has been overexploited and traded illegally, meriting protection measures. Several initiatives to curb illegal activities have been proposed as genetic based methods using population genetics and DNA fingerprinting. In frame of the “Large Scale Project on Genetic Timber Verification” (funded by the German Federal Ministry of Food and Agriculture), this study developed a genetic database of *Handroanthus* sp. with 399 samples from Bolivia, Brazil and French Guyana. The aim of this study was to propose a timber tracking system for Ipê timber to check the origin of *Handroanthus* sp., using SNPs markers and assignment test. Self-assignment and timber assignment tests were performed comparing three genetic approaches: Bayesian method; frequency method; nearest neighbour method. Genotypic diversity (G_d) ranged from 0.3 to 0.8 for 15 out of the 21 populations, indicating the existence of identical genotypes, which may hinders tracking individual trees. However the estimated standardised genetic differentiation among locations (G'_{ST}) was high (0.607) supporting the timber tracking to location. For self-assignment tests, the nearest neighbour method showed the most successful correct assignment rates with 96% at the country level, 85% at the location level and 93% among Brazilian states. Timber assignment tests in country and Brazilian States levels were strongly disturbed by the mix of *Handroanthus* sp. Brazilian *H. impetiginosus* timber samples were assigned mainly to French Guyana, which the reference samples were composed by 19% of *H. impetiginosus* individuals against 9% in the Brazilian reference samples. There is a robust potential to apply genetic based approaches to *Handroanthus* sp. origin detection, nevertheless an increase in the number of *H. impetiginosus* samples in the database is necessary.

Keywords: Bayesian method. DNA fingerprinting. Frequency method. *Handroanthus* sp. Nearest neighbour method. Single nucleotide polymorphism. Timber tracking.

5.1 INTRODUCTION

Rapid deforestation is a fact in most tropical regions, and many tropical forest biomes have been reduced to forest fragments (ASNER et al., 2005; SEBBENN et al., 2008; LACERDA et al., 2013). Selective logging followed by clear-cut and fire is among of the main factors of landscape changes in the Amazon rainforest. The genus *Handroanthus* (formally *Tabebuia*) commonly named as "Ipe" or "Lapacho", which belongs to the botanical family Bignoniacaea, presents one of the most valuable timber from South America. Due to its high-quality timber and high commercial value Ipe has been overexploited and traded illegally. Although *Handroanthus* species are normally not distinguished commercially, species such as *H. impetiginosus* ([Martius ex A. P. de Candolle] J.R. Mattos) and *H. serratifolius* ([Vahl] S. Grose) are considered threatened as a result of the high intensity of exploitation and protection measures should be implemented (SCHULZE et al., 2008). A total of 160.000 cubic meters of sawn Ipe timber were exported from Brazil between 2011 and 2013, the most exported timber in volume and among the most valuable, with an average price of 816 US$ per cubic meter for sawn wood (ITTO, 2014).

It is estimated that the illegal trade represents a total of 30-100 billion dollars per year (THE ECONOMIST, 2012), approximately 50% of the timber sold from the Amazon Basin, Central Africa, Southeast Asia and the Russian Federation (GONÇALVES, 2012). There is a great difficulty and high cost of monitoring the chain of custody (CoC), timber origin and the declared timber species that are been traded.

Several methods have been proposed to control illegal timber trade using physical, chemical and genetic based techniques or even an integration of these approaches (DORMONTT et al., 2015). According to Lowe and Cross (2011), it is between the forest concessions and sawmills that most of the illegally sourced timber tend to be introduced into the legal lots. Thus a technique which cannot be falsified, such as DNA fingerprinting/DNA profiling, would improve significantly the reliability of control systems. The DNA fingerprinting is an intrinsic attribute of all living-being including trees (DEGEN et al., 2013), the genetic information in the logs or sawn timber is the same as the original tree (THE ECONOMIST, 2012; DEGEN et al., 2013). DNA fingerprinting is revolutionizing the identification of individual organisms and their origins, and it has being successfully employed for timber tracking, as for example for *Intsia palembanica* (LOWE et al., 2010). By collecting timber samples along the supply chain, it is possible to detect if there was and at

which point was the insertion of illegal timber into the lots. Timber assignment based on DNA fingerprint have been developed such as in *Swietenia macrophylla* King that obtained successful result in tracking the country of origin of timber pieces (DEGEN et al., 2013). This technique has already been used as certification in Southeast Asia, known as CERTISOURCE (http://www.certisource.co.uk/). Genetic certification of chain of custody based on DNA fingerprint and population genetics are among of the most promising techniques to curb illegal timber trade, and therefore the rapid deforestation and deterioration of the Amazon and other natural forests.

Several multidisciplinary initiatives to curb these illegal activities have been promoted, such as the Global Timber Tracking Network (GTTN). Through the "Large Scale Project On Genetic Timber Verification" (LSP) funded by the German Federal Ministry of Food and Agriculture, the creation of a genetic reference data to check the country of origin of 14 valuable timber species from South America and Africa has been carried out and it will be integrated into the GTTN database.

This study aimed to investigate the possibility to track *Handroanthus* sp. timber based on DNA fingerprinting and using different assignment tests. This method should provide regulatory bodies, operators and timber traders an effective tool to achieve chain of custody control and also provide evidence to prove the declared origin of traded lots.

5.2 MATERIALS AND METHODS

5.2.1 Sampling

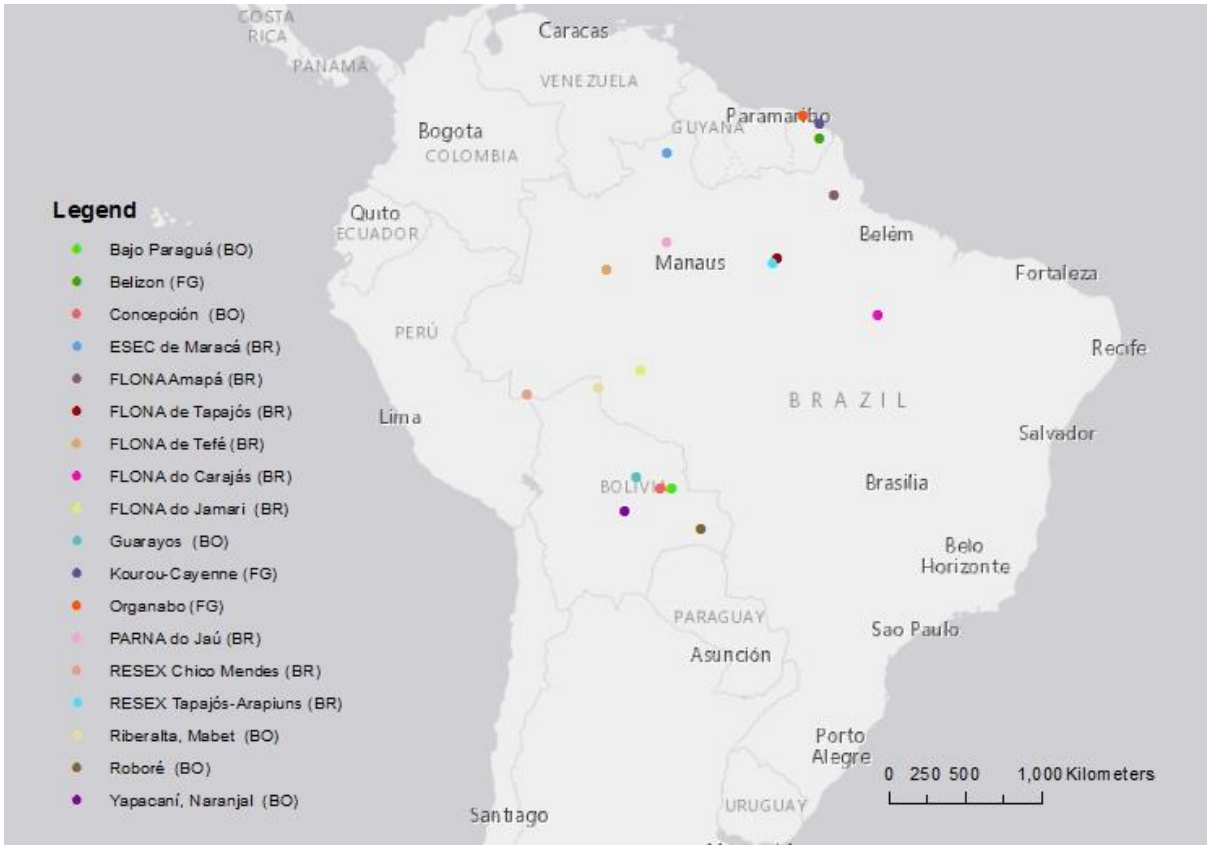
The sampling was carried out in the Amazon rainforest in Bolivia, Brazil and French-Guyana. Cambium and leaf samples of individuals were collected in six locations in Bolivia, nine in Brazil and three in French Guyana (Table 1, Figure 1). In Bolivia the sampling was carried out in farms and forest concessions. In Brazil the sampling was executed in national forests, extractive reserves, ecological stations and national parks with the support of Chico Mendes Institute of Biodiversity (Brazilian governmental institution). In French Guyana the sample collection was carried by the Institut National de la Recherche Agronomique- INRA together with the forest authorities (Office National des Forêts, ONF). Leaf or cambium tissue were collected from 5 to 38 trees in each location and stored in a labelled plastic bag with silica gel.

Table 1- Location, species and samples size of *Handroanthus* sp. sample-populations of cambium samples used in this study.

Country	Sample-populations	Species	Latitude	Longitude	Sample size
Bolivia	Bajo Paraguá	*H. serratifolius*	-16.0912	-61.1504	20
Bolivia	Concepción	*H. serratifolius*	-16.1681	-61.7821	19
Bolivia	Guarayos	*H. serratifolius*	-15.5095	-63.1971	20
Bolivia	Riberalta, Mabet	*Handroanthus* sp.	-10.3637	-65.4438	13
Bolivia	Roboré	*H. serratifolius*	-18.4391	-59.4357	23
Bolivia	Yapacaní, Naranjal	*H. serratifolius*	-17.4311	-63.9210	20
Brazil	FLONA do Jamari	*H. impetiginosus*	-9.3983	-62.9248	20
Brazil	FLONA do Jamari	*H. incanus*	-9.3924	-62.9224	5
Brazil	FLONA do Jamari	*H. serratifolius*	-9.4051	-62.9213	6
Brazil	FLONA de Tefé	*Handroanthus* sp.	-3.5245	-64.9738	14
Brazil	ESEC de Maracá	*Handroanthus* sp.	3.3739	-61.4497	21
Brazil	FLONA Amapá	*Handroanthus* sp.	0.8422	-51.5999	22
Brazil	FLONA de Tapajós	*H. serratifolius*	-2.8655	-54.9284	20
Brazil	FLONA do Carajás	*H. serratifolius*	-6.1972	-49.0847	32
Brazil	PARNA do Jaú	*Handroanthus* sp.	-1.9047	-61.4387	22
Brazil	Resex Chico Mendes	*Handroanthus* sp.	-10.7810	-69.6444	38
Brazil	Resex Tapajós - Arapins	*Handroanthus* sp.	-3.0788	-55.2837	21
French Guyana	Belizon	*H. serratifolius*	4.2120	-52.5249	8
French Guyana	Belizon	*H. impetiginosus*	4.2082	-52.5520	12
French Guyana	Paracou, Kourou-Cayenne	*H. serratifolius*	5.0231	-52.5196	25
French Guyana	Organabo	*H. serratifolius*	5.5399	-53.4585	18

Source: author of this study.

Figure 1- Cambium and leaf sampling locations



Source: author of this study.

For each sampled tree (for both fresh or timber samples) a unique tree number was given, GPS coordinates (longitude and latitude), diameter at breast height (at 1.3 m height), botanical name of the species/genus and date of collection were noted. In same locations the individuals were identified in the genus level due to similarity of some *Handroanthus* sp. species.

A similar procedure was executed for timber samples collection. A total of 20 timber samples were collected after the falling of the tree at the Gleba Mamuru-Arapiuns (Brazil) in 2013, at the concession area manage by the company LN Guerra (Table 2).

Table 2- Individual timber code, species and location of *Handroanthus* sp. sampled in Gleba Mamuru-Arapiuns region, Brazil.

Samples code	Species	latitude	Longitude
T046	*H. impetiginosus*	-2.846015	-56.034271
T047	*H. impetiginosus*	-2.846219	-56.034366
T048	*H. serratifolius*	-2.845817	-56.034568
T055	*H. serratifolius*	-2.845617	-56.032481
T057	*H. serratifolius*	-2.846022	-56.038429
T058	*H. impetiginosus*	-2.846372	-56.037666
T098	*H. impetiginosus*	-2.832560	-56.056366
T104	*H. impetiginosus*	-2.831829	-56.054514
T105	*H. impetiginosus*	-2.832750	-56.054919
T112	*H. impetiginosus*	-2.833705	-56.051563
T126	*H. impetiginosus*	-2.827449	-56.048593
T128	*H. serratifolius*	-2.827085	-56.052130
T140	*H. impetiginosus*	-2.831333	-56.058377
T167	*H. impetiginosus*	-2.830550	-56.061232
T188	*H. impetiginosus*	-2.826638	-56.061781
T194	*H. impetiginosus*	-2.826567	-56.063621
T203	*H. impetiginosus*	-2.836028	-56.065877
T216	*H. impetiginosus*	-2.830381	-56.061905
T223	*H. impetiginosus*	-2.831845	-56.066102
T229	*H. impetiginosus*	-2.830622	-56.065569

Source: author of this study.

5.2.2 DNA analysis

Cambium samples collected in Brazil were sent the Laboratory of Population Genetics and Forestry of São Paulo State University (UNESP), School of Engineering, Ilha Solteira-Brazil, for DNA isolation. Timber samples from Brazil and cambium samples collected in Bolivia and French Guyana were sent to Thünen Institute of Forest Genetics facilities in Großhansdorf, Germany, for DNA isolation. DNA isolation from leaf and cambium was carried out according to Dumolin et al. (1995), while DNA extraction of timber was conducted as described by Lowe et al. (2015).

The total of 399 samples were screened for 121 SNP and INDEL markers (described in the Chapter 2) using the MassARRAY® iPLEX™ genotyping. A final selection of 68 SNPs/INDELs markers with minimum amplification rate of 95% were selected for genetic tracking analysis.

5.2.3 Data analysis

5.2.3.1 Genotypic diversity, genetic diversity and differentiation

Genetic diversity within sample-populations was quantified for nuclear markers using GDA_NT software (DEGEN, unpublished) by the following indices: percentage of polymorphic loci (P); mean observed heterozygosity ($H_o = 1 - \sum_{i=1}^{k} p_i$, where p_i is the allele frequency in the allele i, k is the number of alleles in the locus); mean expected heterozygosity ($H_e = 1 - \sum p_{ii} = pij = \frac{\sum_{i \neq j} nij}{n}$, where p_{ii} is the frequency of homozygote genotypes, p_{ij} is the frequency of heterozygotes genotypes, n_{ij} is the number of heterozygotes individuals and n is the samples size); and fixation index ($F = 1 - (H_o / H_e)$), total number of alleles (N_a) and genotypic diversity (G_d) within sample-populations:

$$G_d = \frac{G-1}{n-1},$$

where G is the number of distinct multilocus genotypes and n is the total number of individuals sampled.

Genetic differentiation and fixation measure among sample-populations, as well as among contries for the 68 loci were calculated using Wright's F_{ST} -statistic (WEIR, 1990), standardised G'_{ST} -statistic (HEDRICK, 2005) and δ_G -statistic (GREGORIUS; DEGEN; KOENIG, 2007).

5.2.3.2 Genotype assignment test

Assignment tests were performed using three different approaches: Bayesian multilocus-approach (BM) (RANNALA; MOUNTAIN, 1997); frequency method (FM) (PAETKAU et al., 1995); and the nearest neighbour approach (NNA) (DEGEN et al., 2017); all methods were associated to exclusion probabilities for the timber assignment test. Bayesian multilocus-approach and the frequency method methods calculate the allele frequencies for all loci of the reference populations and the probability of the multilocus genotype of the tested individual to be generate from this population/group, assigning the tested individual to the population with the presenting the highest probability. Both approaches are based in allele frequency and assume Hardy-Weinberg-Proportions, linkage

equilibrium or completely unlinked loci (DEGEN et al., 2017). However, several species have demonstrated that these principles are not applicable to natural populations, for example due to apomixes in *H. serratifolius* (ALVES et al., 2016). The method of the nearest neighbour presents an alternative to allele frequency based methods by assigning samples based on the genetic distance between individuals. The nearest neighbour method computes the multilocus genotypes genic distance between a tested individual and each reference individuals, being the nearest neighbours those presenting the smallest distance to the tested sample. An index (I_r) which weights the proportion of individuals from a group among the nearest neighbours in relation to the proportion of this group among all reference populations is calculated to supress the sample size effect (DEGEN et al., 2017). The I_r varies from -1 to 1; -1 represents groups that don't contain nearest neighbours and 1 groups that contains all nearest neighbours. The tested sample is assigned to the population which contains the highest proportion of nearest neighbours. This approach seems to work better in imperfect grouping of reference samples, such as the species mixed (only genus confirmed) populations used in this study (DEGEN et al., 2017).

Self-assignment tests, using the leave-one-out approach, were carried out in location, country and Brazilian states levels. For the country level all 399 samples were used for the analysis. For the location level analysis only sample-populations that presented at least 20 individuals were taken into account, totalling 13 sample-populations analysed. For the analysis in level of Brazilian States, 221 samples from Brazil were pooled into state groups (Acre, Amapá, Amazonas, Pará, Rondonia and Roraima). The assignment of the timber samples to country and Brazilian states were equally carried out using the same three different assignment tests.

5.3 RESULTS

5.3.1 Genetic diversity and genetic differentiation

The percentage of polymorphic loci (P) ranged among locations from 8.1 to 91.9%, with a mean among locations of 49.9% (Table 3). The total number of alleles (N_a) over all 68 SNPs loci analysed ranged among locations from 73 to 117 alleles, with a mean among locations of 94.2. In 15 of the 21 sample-populations, identical multilocus genotypes were detected, indicating a genotypic diversity (G_d) ranging from 0.27 to 1.0, with mean of 0.68.

The mean observed heterozygosity (H_o) ranged from 0.01 to 0.49 (mean of 0.15), expected heterozygosity (H_e) ranged from 0.01 to 0.32 (mean of 0.13). In four of the 21 sample-populations there was a significant excess of heterozygous individuals in comparison with expected by Hardy-Weinberg equilibrium (HWE) expectations, with fixation index (F) ranging from -0.24 to -0.61, and in six sample-populations there was a significant excess of homozygous individuals, with fixation index (F) ranging from 0.15 to -0.23, and indication inbreeding.

Table 3- Genetic diversity for species location: n is the sample size; P is the percentage of polymorphic loci; N_a is the total number of alleles; G_d is the genotypic diversity; H_o is the observed heterozygosity; H_e is the expected heterozygosity; F is the fixation index (F).

Country	**Location**	**Species**	n	P (%)	N_a	G_d	H_o	H_e	F
Bolivia	Bajo Paraguá	*H. serratifolius*	20	75.7	113	0.63	0.09	0.12	0.23*
Bolivia	Concepción	*H. serratifolius*	19	16.2	77	0.50	0.05	0.04	-0.03
Bolivia	Guarayos	*H. serratifolius*	20	81.1	110	1.00	0.39	0.28	-0.32*
Bolivia	Riberalta-Mabet	*Handroanthus* sp.	13	64.9	99	0.78	0.08	0.09	0.09
Bolivia	Roboré	*H. serratifolius*	23	67.6	101	0.59	0.08	0.09	0.15*
Bolivia	Yapacaní- Naranjal	*H. serratifolius*	20	70.3	95	0.44	0.49	0.29	-0.61*
Brazil	FLONA do Jamari	*H. impetiginosus*	20	16.2	77	0.50	0.03	0.03	-0.03
Brazil	FLONA do Jamari	*H. incanus*	5	62.2	100	1.00	0.35	0.23	-0.44
Brazil	FLONA do Jamari	*H. serratifolius*	6	8.1	73	0.67	0.02	0.02	0.14
Brazil	FLONA de Tefé	*Handroanthus* sp.	14	8.1	79	0.50	0.01	0.01	0.02
Brazil	ESEC de Maracá	*Handroanthus* sp.	21	78.4	112	1.00	0.14	0.19	0.17*
Brazil	FLONA Amapá	*Handroanthus* sp.	22	86.5	116	1.00	0.40	0.30	-0.24*
Brazil	FLONA de Tapajós	*H. serratifolius*	20	91.9	117	0.81	0.14	0.19	0.19*
Brazil	FLONA do Carajás	*H. serratifolius*	32	81.1	109	0.95	0.25	0.29	0.09
Brazil	PARNA do Jaú	*Handroanthus* sp.	22	10.8	80	0.43	0.01	0.02	0.21*
Brazil	RESEX Chico Mendes	*Handroanthus* sp.	38	18.9	78	0.44	0.04	0.03	0.05
Brazil	RESEX Tapajós-Arapins	*Handroanthus* sp.	21	13.5	74	0.47	0.03	0.03	0.23*
French Guyana	Belizon	*H. serratifolius*	8	13.5	75	0.43	0.05	0.05	0.08
French Guyana	Belizon	*H. impetiginosus*	12	73.0	112	0.27	0.07	0.09	0.21
French Guyana	Paracou-Kourou-Cayenne	*H. serratifolius*	25	89.2	104	1.00	0.45	0.32	-0.35*
French Guyana	Organabo	*H. serratifolius*	18	21.6	77	0.83	0.07	0.06	-0.09
Mean			19	49.9	94.2	0.68	0.15	0.13	-0.01

P< 0.05; Source: author of this study.

All estimates of genetic differentiation for nuclear, CpDNA, MtDNA and over all loci among both locations and countries (see suplementary material S1 for table with details per locus), based on Gregorius's delta (δ_G), Wright's F_{ST}-statistic and Hedrick standardised G'_{ST} were significant higher than zero (Table 4), indicating population genetic structure. The mean genetic differentiation over all loci among both locations and countries based on Gregorius's

delta (δ_G) were 0.22 and 0.15, respectively. Wright's F_{ST} and standardised G'_{ST} were higher among locations (F_{ST} = 0.537 and G'_{ST} = 0.607) than among countries (F_{ST} = 0.121 and G'_{ST} = 0.171). All δ_G values estimated among locations for nuclear, CpDNA, MtDNA and over all loci (0.201-0.313) were lower than F_{ST} (0.355-0.879) and G'_{ST} (0.437-0.923). Among contries, all δ_G values estimated for the samples (0.095-0.235) were lower than F_{ST} (0.053-0.223) and as well as, for nuclear marker (δ_G = 0.95) than G'_{ST} (0.079). All δ_G, F_{ST} and G'_{ST} values estimated for nuclear markers among locations (0.201-0.437) and countries (0.053-0.095) were lower than estimated for CpDNA and MtDNA among locations (0.208-0.923) and countries (0.186-0.316).

Table 4- Mean genetic differentiation among locations and among countries. n_l is the number of loci; δ_G is the Gregorius δ_G-statistic; F_{ST} is the Wright's fixation index; G'_{ST} is the Herdick standardised G'_{ST}-statistic.

		Among locations			**Among countries**		
Mean	n_l	δ_G	F_{ST}	G'_{ST}	δ_G	F_{ST}	G'_{ST}
Nuclear	37	0.201**	0.355**	0.437**	0.095**	0.053**	0.079**
Cp-DNA	13	0.313**	0.879**	0.923**	0.235**	0.223**	0.316**
MT-DNA	18	0.208**	0.665**	0.729**	0.202**	0.186**	0.255**
Cp-MT-DNA	31	0.252**	0.755**	0.810**	0.216**	0.202**	0.281**
All	68	0.224**	0.537**	0.607**	0.150**	0.121**	0.171**

** P< 0.01. Source: author of this study.

5.3.2 Genotype self-assignment tests

The self-assignment test in the country level obtained a success of 72% with the Bayesian approach, 74% with the frequency method and 96% with the nearest neighbour (Table 5). The minimum success rate for the nearest neighbour method was 95%, while for Bayesian approach was 84% and 85% with the frequency method.

Table 5- Percentile of correct self-assignment samples in country level by employing the Bayesian method, the frequency method and the nearest neighbour approach.

Country	Sampling size	Bayesian	Frequency	Nearest neighbour
Bolivia	115	84%	85%	95%
Brazil	221	92%	95%	95%
French Guyana	63	41%	41%	100%
Mean		72%	74%	96%

Source: author of this study.

Self-assignment performed in location level used only sample-populations with 20 or more individuals: Bajo Paraguá Guarayos, Roboré and Yapacaní-Naranjal in Bolivia; FLONA do Jamari for *H. impetiginosus*, ESEC de Maracá, FLONA Amapá, FLONA de Tapajós, FLONA do Carajás, PARNA do Jaú, Resex Chico Mendes and Resex Tapajós-Arapiuns in Brazil; and Paracou-Kourou-Cayenne in French Guyana. The successes achieved for the self-assignment were of 77% with the Bayesian approach, the 79% with the frequency method and 85% with the nearest neighbour approach (Table 6).

Table 6- Percentile of correct self-assignment samples in location level by employing the Bayesian, frequency and the nearest neighbour method.

Location	Sampling size	Bayesian	Frequency	Nearest neighbour
Bajo Paraguá (BO)	20	0%	0%	60%
Guarayos (BO)	20	80%	80%	80%
Roboré (BO)	23	96%	87%	48%
Yapacaní, Naranjal (BO)	20	100%	100%	100%
FLONA do Jamari (BR)	20	100%	100%	95%
ESEC de Maracá (BR)	21	86%	95%	95%
FLONA do Amapá (BR)	22	73%	95%	95%
FLONA do Tapajós (BR)	20	5%	5%	40%
FLONA do Carajás (BR)	32	72%	72%	91%
PARNA do Jaú (BR)	22	100%	100%	100%
RESEX Chico Mendes (BR)	38	95%	95%	95%
RESEX Tapajós-Arapiuns (BR)	21	100%	95%	95%
Paracau, Kourou-Cayenne (FG)	25	100%	100%	100%
Mean		77%	79%	85%

Source: author of this study.

The self-assignment test performed for Brazilian states level pooled together sample-populations belonging to the same state of origin: Acre (RESEX Chico Mendes), Amapá (FLONA do Amapá), Amazonas (FLONA de Tefé and PARNA do Jaú), Pará (FLONA de Tapajós, FLONA do Carajás and RESEX Tapajós-Arapiuns), Rondônia (FLONA do Jamari with all species) and Roraima (ESEC de Maracá). A total of 221 samples were analysed and

the percentage of successfully self-assigned samples were of 87% with the Bayesian approach, the 89% with the frequency method and 93% with the nearest neighbour approach (Table 7).

Table 7- Percentile of correct self-assignment samples among Brazilian states level by employing the Bayesian, frequency and the nearest neighbour method.

Brazilian States	Sampling size	Bayesian	Frequency	Nearest neighbour
Amazonas	36	100%	100%	100%
Roraima	21	86%	95%	95%
Amapá	22	95%	95%	95%
Pará	73	81%	84%	92%
Rondonia	31	65%	65%	84%
Acre	38	97%	95%	95%
Mean		87%	89%	93%

Source: author of this study.

5.3.3 Timber assignment test

The following analyses were developed by assigning individual timber samples to the country and Brazilian states reference levels, separately. Due to the age of the timber samples used (~3 years) the amplification rate was lower (67.3%) in comparison with cambium and leaf material (over 95%), with only one locus amplified for all 20 samples (tas0000846). Samples with less than 40% of analysable data (27 loci) were excluded from the analysis and a final number of eight samples were tested. In the country level the Bayesian and frequency methods achieved similar results, both with 100% of correct assignment to Brazil (Table 8). All score-values were significant (P> 0.99), except for the sample T194 that presented non-significant score-values with the Bayesian (P= 0.688) and frequency (P= 0.683) method. The nearest neighbourhood method differently from the others two methods obtained a not significant index (I_r = 38%) for samples assigned to Brazil (Table 8). All samples assigned to French Guyana with a significant index (I_r) were *H. impetiginosus* samples (Table 2)

The assignment test in Brazilian states level (Table 9) presented similar results as those obtained for country level assignment. *H. impetiginosus* timber samples were, for all three methods, mainly assigned to Rondônia that is the Brazilian state that presents declared *H. impetiginosus* samples. Timber samples were also assigned to the Amazonas state and Pará (correct state of origin). The samples T048 (*T. serratifolius*) and T229 (*H.impetiginosus*) were assigned by the three methods as being from Amazonas state (significant with the

probability> 0.95) with low exclusion probabilities. The third sample (T194- *H. impetiginosus*) assigned to Amazonas state with the Bayesian and frequency methods presented a high score (BM: 0.995; FM: 1.000), however it also obtained a high exclusion probability significant with probability > 0.95. This sample was significantly (P< 0.99) assigned with the nearest neighbourhood approach to Rondônia with an index of 1.000 and not significant exclusion probability with at 0.90, presumably due to the species.

Table 8- Assignment for timber samples among countries, using the Bayesian, frequency and nearest neighbour methods. n_l is the number of loci; Prob: probability of exclusion based on LOD1-values; E_p is the exclusion probability based on outlier genotypes; I_r is the proportion index.

		Bayesian method				**Frequency method**				**Nearest neighbourhood method**		
Sample	n_l	**Assigned country**	**LOG(L)**	**Score**	**Prob**	**Assigned country**	**LOG(L)**	**Score**	**Prob**	**Assigned country**	E_p	I_r
T048	30	Brazil	5.57	0.999	0.091	Brazil	5.56	0.999	0.089	F. Guyana	0.467	0.286
T055	36	Brazil	9.70	0.992	0.626	Brazil	9.70	0.993	0.641	Brazil	0.621	0.250
T128	44	Brazil	9.41	1.000	0.241	Brazil	9.39	1.000	0.238	Brazil	0.573	0.750
T140	37	Brazil	8.26	0.994	0.427	Brazil	8.26	0.995	0.451	F. Guyana	0.489	1.000***
T188	44	Brazil	11.17	0.996	0.535	Brazil	11.18	0.996	0.503	F. Guyana	0.607	1.000***
T194	32	Brazil	9.06	0.688	0.562	Brazil	9.07	0.683	0.555	F. Guyana	0.697	0.714***
T216	41	Brazil	10.04	0.996	0.409	Brazil	10.04	0.997	0.448	F. Guyana	0.620	1.000***
T229	35	Brazil	5.48	0.998	0.028	Brazil	5.46	0.998	0.025	Brazil	0.430	0.500

*** P<0.001; Source: author of this study.

Table 9- Assignment for timber samples among Brazilian states by employing the Bayesian method, the frequency method and the nearest neighbour approach. Prob: probability of exclusion based on LOD1-values; Ex. Prob: exclusion probability based on outlier genotypes; Ir: proportion index.

		Bayesian method				**Frequency method**				**Nearest neighbourhood method**		
Sample	n_l	**Assigned state**	**-LOG(L)**	**Score**	**Prob**	**Assigned state**	**-LOG(L)**	**Score**	**Prob**	**Assigned state**	**Prob**	**I_r**
T048	30	Amazonas	1.99	0.988	0	Amazonas	1.87	0.989	0.12	Amazonas	0.644	1.000 ***
T055	36	Rondônia	10.57	0.572	0.997	Rondônia	11.32	0.495	0.997	Para	0.641	0.333 ^{ns}
T128	44	Pará	9.45	1.000	0.472	Pará	9.44	1.000	0.430	Para	0.609	1.000 **
T140	37	Rondônia	5.43	0.994	0.666	Rondônia	5.41	0.999	0.683	Rondonia	0.623	0.800 ***
T188	44	Rondônia	8.16	1.000	0.774	Rondônia	8.19	1.000	0.773	Amazonas	0.681	0.667 *
T194	32	Amazonas	6.41	0.995	0.973	Amazonas	6.55	1.000	0.978	Rondonia	0.729	1.000 ***
T216	41	Rondônia	7.87	0.983	0.837	Rondônia	7.95	0.988	0.794	Rondonia	0.698	0.889 ***
T229	35	Amazonas	3.00	0.970	0.333	Amazonas	2.88	0.971	0.320	Amazonas	0.622	0.500 *

*** P< 0.01; ** P< 0.1. Source: author of this study.

5.4 DISCUSSION

Illegal timber trade, either in the species context as well as illegally sourced has being a major problem in tropical forest. This studies show the potential of the DNA fingerprint to track origin, as well as to follow and verify the chain of custody of timber products.

5.4.1 Genetic diversity and genetic differentiation

The results in genotypic diversity was low in some sample-populations, due to the existence of identical genotypes within population. In the Bolivian location of Yapacaní-Naranjal a high number of identical genotypes were detected with a low genotypic diversity value of 0.4, even though presenting an excess of heterozygotes with a fixation index of -0.606, indicating heterozygotes identical genotypes. The sample-populations of Yapacaní-Naranjal (Bolivia), Belizon- *H. impetiginosus* (French Guyana) and Bajo Paraguá in (Bolivia) had low genotypic diversity (G_d) of 0.4, 0.3 and 0.6, respectively, although presenting relative high percentage of polymorphic loci of 70.3, 73.0 and 75.7%. Similar results were obtained in four *Prunus avium* populations in Germany, species which reproduces through clonal propagation and outcrossing (JOLIVET et al., 2011). *Handroanthus serratifolius* is recorded as practicing sexual as well as apomixis in some populations in the Brazilian savannah (ALVES et al., 2016), fact which could explain the high number of identical genotypes.

Wright's F_{ST} and standardised G'_{ST} obtained were greater among populations than among countries as consequence of group/populations-specific alleles among populations be more common than among countries. Despite the relavively low variation of Gregorius's delta (δ_G), G'_{ST} among population (G'_{ST} = 0.607) shows that there are relatively high differenciation in terms of populations-specific alleles, thus supporting a strong for determining population of origin.

5.4.2 Genotype self-assignment tests

The results obtained show a high power of correct assignment in all three levels: among countries, locations and Brazilian states. All three levels attained the higher success rate of correct self-assignment by employing the nearest neighbour approach with 96% among

countries, 85% among locations and 93% among Brazilian states. This approach confirmed the success expectation in imperfect grouping of reference samples.

At the location level, the sample-population of Bajo Paraguá in Bolivia presented a 0% of corrected self-assignment rate for both allele frequency based methods (Bayesian and Frequency methods); nevertheless its samples were assigned to 90% to Roboré and 10% to Yapacaní-Naranjal, both in Bolivia. Self-assignment of samples to Brazilian states, Rondônia state had the major percentage of incorrect assignments for all three approaches. This occurred possibly due to the mix of species, due to the fact that *H. impetiginosus* and *H. incanus* might occur in other locations that were botanically identified only in the genus level.

5.4.3 Timber assignment tests

In the analysis in the country level most of the timber samples were incorrectly assigned to French Guyana. This fact can be explained by the mixed species of the timber samples and sample-populations with *H. impetiginosus*, *H. incanus* and *H. serratifolius*. Nineteen percent of the samples from French Guyana (12 individuals) are *H. impetiginosus*, against 9% of the Brazilian samples (20 individuals) (|Table1), associated with the small number of individuals from French Guyana (63) in comparison with Brazil (221) and Bolivia (115) (Table 1).

5.5 CONCLUSION

For timber samples it's strongly recommended more repetitions of the DNA extractions before MassARRAY® iPLEX™ genotyping to obtain better amplification rates.

The occurrence of identical genotypes hinders the possibility of controlling individual trees, however no disturbing the chain of custody control in the location level, supported by the high differentiation of standardised G'_{ST} among locations.

Self-assigment tests were highly effective, with high correct assigment rates specially with the nearest neighbour approach, supporting the feasibility of a genetic based control. However, a better descrimination of species, as well as a greater database for others *Handroanthus* species, such as *H. impetiginosus*, is necessary to increace geographical assigment accuracy.

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SUPPLEMENTARY MATERIAL

S1: Mean genetic differentiation among populations and among countries. δ_G is the Gregorius δ_G-statistic; F_{ST} is the Wright's fixation index; G'_{ST} is the Herdick standardised G'_{ST}-statistic.

(continue)

	Among populations			Among countries		
Locus	δ_G	F_{ST}	G'_{ST}	δ_G	F_{ST}	G'_{ST}
tas0000082	0.2485	0.3628	0.4755	0.0455	0.0074	0.0124
tas0000119	0.1128	0.2839	0.3164	0.0839	0.0740	0.0849
tas0000134	0.0752	0.1960	0.2135	0.0383	0.0206	0.0230
tas0000211	0.0659	0.1412	0.1554	0.0643	0.0442	0.0515
tas0000294	0.2334	0.3066	0.4050	0.0417	0.0064	0.0105
tas0000443	0.0733	0.2311	0.2477	0.0822	0.0695	0.0802
tas0000496	0.0999	0.3736	0.4050	0.1398	0.1305	0.1602
tas0000513	0.0770	0.1287	0.1432	0.0359	0.0139	0.0161
tas0000541	0.0902	0.2999	0.3273	0.0730	0.0637	0.0720
tas0000637	0.3937	0.6568	0.7902	0.0922	0.0220	0.0466
tas0000838	0.2482	0.3863	0.5047	0.1248	0.0449	0.0785
tas0000846	0.3158	0.5120	0.6711	0.2528	0.1471	0.3013
tas0000880	0.3344	0.4854	0.6415	0.1352	0.0439	0.0925
tas0000984	0.2205	0.3273	0.4240	0.0546	0.0127	0.0205
tas0000993	0.1969	0.5684	0.6445	0.2162	0.1606	0.2687
tas0001084	0.1799	0.2071	0.2787	0.1056	0.0418	0.0642
tas0001089	0.2750	0.3792	0.5049	0.0569	0.0098	0.0176
tas0001118	0.0800	0.1148	0.1325	0.0331	0.0128	0.0147
tas0001119	0.2913	0.3977	0.5827	0.1008	0.0203	0.0496
tas0001135	0.0897	0.2583	0.2812	0.0916	0.0754	0.0890
tas0001208	0.3938	0.6438	0.7823	0.0505	0.0081	0.0168
tas0001231	0.1998	0.3906	0.4734	0.0848	0.0313	0.0447
tas0001289	0.3826	0.7565	0.8483	0.2292	0.1197	0.2336
tas0001361	0.1151	0.4017	0.4347	0.1297	0.1164	0.1425
tas0001399	0.2609	0.3641	0.4796	0.0674	0.0171	0.0293
tas0001470	0.2427	0.7383	0.7986	0.3377	0.4018	0.5251
tas0001562	0.1383	0.1694	0.2152	0.0415	0.0112	0.0152
tas0001653	0.0956	0.2227	0.2557	0.0459	0.0170	0.0219
tas0001740	0.2378	0.3760	0.4817	0.0753	0.0203	0.0318
tas0001766	0.1376	0.1061	0.1944	0.0763	0.0123	0.0301
tas0001767	0.3245	0.4817	0.6284	0.0546	0.0094	0.0182
tas0001796	0.1122	0.2014	0.2354	0.0776	0.0403	0.0515
tas0001816	0.2550	0.3795	0.5304	0.1031	0.0271	0.0552
tas0001827	0.2866	0.4724	0.5955	0.1231	0.0487	0.0865
tas0001921	0.2923	0.4581	0.5976	0.0943	0.0296	0.0546

S1: Mean genetic differentiation among populations and among countries. δ_G is the Gregorius δ_G-statistic; F_{ST} is the Wright's fixation index; G'_{ST} is the Herdick standardised G'_{ST}-statistic.

(conclusion)

	Among populations			Among countries		
Locus	δ_G	F_{ST}	G'_{ST}	δ_G	F_{ST}	G'_{ST}
tas0001943	0.0265	0.0394	0.0411	0.0077	0.0015	0.0016
tas0001945	0.2365	0.3122	0.4153	0.0602	0.0131	0.0219
tasCp20_12116	0.4982	0.8881	0.9443	0.3286	0.2478	0.4324
tasCp20_16837	0.4747	0.8100	0.9020	0.2081	0.1088	0.2354
tasCp229_2616	0.2784	0.9265	0.9472	0.2536	0.2861	0.3681
tasCp229_8690	0.3329	0.7868	0.8558	0.2186	0.1679	0.2680
tasCp373_68	0.1538	1.0000	1.0000	0.2688	0.3106	0.3996
tasCp373_606	0.2784	0.9265	0.9472	0.2456	0.2800	0.3571
tasCp436_3740	0.3360	0.7983	0.8648	0.2194	0.1677	0.2681
tasCp436_10452	0.2784	0.9265	0.9472	0.2456	0.2800	0.3571
tasCp544_19217	0.3334	0.7925	0.8601	0.2190	0.1678	0.2680
tasCp544_19813	0.3947	0.7025	0.8180	0.0841	0.0172	0.0380
tasCp612_1890	0.2784	0.9265	0.9472	0.2456	0.2800	0.3571
tasCp784_4541	0.1538	1.0000	1.0000	0.2751	0.3190	0.4112
tasCpIN544_750	0.2795	0.9489	0.9633	0.2398	0.2724	0.3465
tasMt53_18528	0.1758	0.1172	0.2612	0.1415	0.0176	0.0524
tasMt79_14230	0.1742	0.6915	0.7417	0.1458	0.1417	0.1733
tasMt79_21228	0.1846	0.7840	0.8200	0.2631	0.2737	0.3667
tasMt173_2622	0.2431	0.6788	0.7533	0.0929	0.0280	0.0461
tasMt615_5988	0.4096	0.7036	0.8268	0.4399	0.4879	0.7389
tasMt680_8937	0.0738	0.4601	0.4800	0.1270	0.1356	0.1589
tasMt732_3426	0.3302	0.6409	0.7602	0.1665	0.0651	0.1300
tasMt967_4329	0.1906	0.5991	0.6676	0.1340	0.1097	0.1399
tasMt1438_1478	0.3042	0.6694	0.7663	0.1630	0.1057	0.1568
tasMt1580_4320	0.1823	0.7320	0.7768	0.2600	0.2640	0.3572
tasMt1751_2224	0.1846	0.7840	0.8200	0.2631	0.2737	0.3667
tasMt2011_567	0.1532	0.8509	0.8741	0.1362	0.1462	0.1728
tasMt2580_2831	0.1846	0.7840	0.8200	0.2567	0.2652	0.3548
tasMt4446_1048	0.1823	0.7320	0.7768	0.2537	0.2559	0.3454
tasMt4446_1728	0.1744	0.6937	0.7435	0.1447	0.1407	0.1719
tasMt6079_1485	0.2281	0.5278	0.6297	0.1315	0.1005	0.1377
tasMtIN173_1921	0.1822	0.7300	0.7751	0.2595	0.2623	0.3555
tasMtIN2369_2284	0.1846	0.7840	0.8200	0.2631	0.2737	0.3667
MEAN	0.2243	0.5372	0.6068	0.1503	0.1206	0.1711

Source: author of this study.