



Applied Genetics for Forest Conservation and Sustainable Trade

Report July 2011

The state of DNA technology for trees & wood products



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Acknowledgements

This report is the result of constructive dialogue at conferences and meetings over a number of years. Double Helix Tracking Technologies Pte Ltd (DoubleHelix) was founded in June 2008, assembled from a team that had been working across different organisations and disciplines, but with a shared vision of applying genetics to forests and the timber supply chain.

It has been a process of discovery; defining the sorts of questions that science could answer, the sorts of questions that businesses, NGOs or conservation investors would ask and then redefining the science. This iterative process is far from over, but this report represents a consolidation of the state that applied genetics is in today with pointers towards its possible development in the future. As such we would like to gratefully acknowledge the input, questioning, feedback, constructive criticism and support from the following:

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Rainforest Alliance	World Wildlife Fund for Nature



Kevin Hill
Founder

Introduction

Applied genetics for forest conservation and sustainable trade is still very much in its infancy. Despite this it is possible to discern distinct future directions which we have used as the framework for this report. We have split the genetic applications into three disciplines; population genetics, DNA fingerprinting and DNA barcoding. Between them they have profound implications for chain-of-custody, certification systems, new legislation, CITES, forest health, reforestation, conservation and beyond.

This report sets out to introduce the technology and how it is being used so that the future implications can be clearly understood. We use examples of projects that are currently under way and introduce projects that are being planned. Next year we look forward to reporting on the progress of the projects and updates on scientific developments.


We have not restricted the content of this report to DoubleHelix's work; in fact we consider an open-source approach as essential to building momentum in this sector and making the inevitable happen sooner rather than later.

The impetus for this report was generated from a very constructive week in Washington, D.C. in May 2011. The week started with meetings at the Smithsonian to discuss a global project to give every species of tree on the planet a DNA barcode, followed by the Potomac Forum hosted by the World Bank and ending with a conference hosted by the Forest Legality Alliance held at the World Resources Institute. Throughout the week we met with various departments of the U.S. Government and were met with well informed, critical and constructive thinking.

We encourage more of the same and have started a blog on this report at this URL:

<http://www.doublehelixtracking.com/company/report>

We look forward to your thoughts, input and contribution to the continued development of this transformational approach to forest conservation and sustainable trade.



Jonathan Geach
Director



Darren Thomas
Director

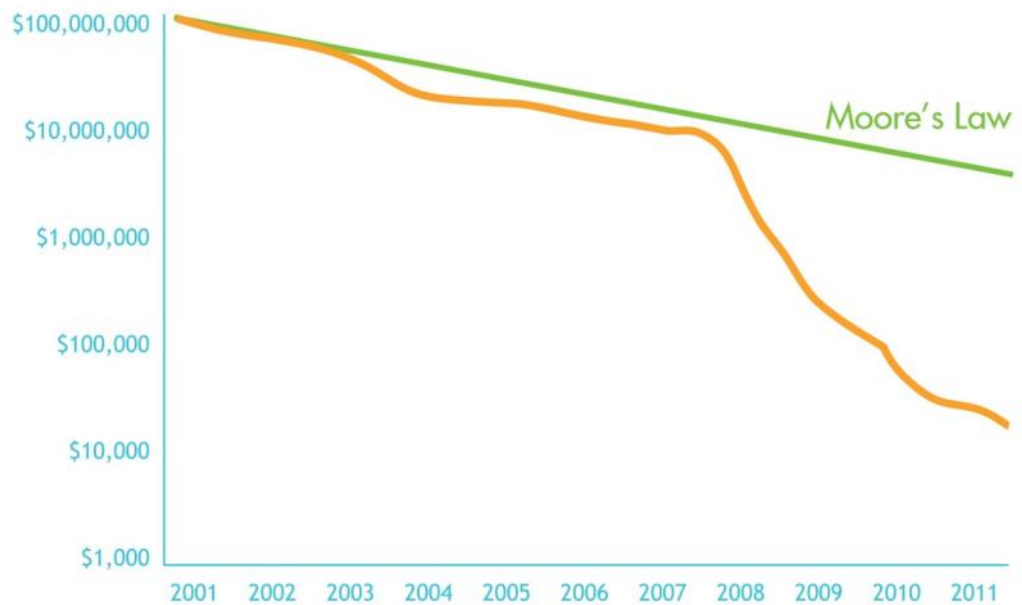
In a world where the genetic information of trees is freely available, those responsible for forest ecosystems are empowered in efforts to preserve habitats and better adapt to climate change.

Double Helix Tracking Technologies (DoubleHelix) is a leader in applied forest genetics for long term conservation and sustainable trade – bridging the gap between cutting edge genetic research, forest management and timber supply chain issues.

Background

Plummeting costs and technological breakthroughs mean that the time has come for applied genetics to make a profound impact on the way we manage and protect forest resources.

Figure 1
Sequencing cost per genome (USD)



Source: National Human Genome Research Institute www.genome.gov/sequencingcosts/

The human genome project was the equivalent of the space race for genetics. It took ten labs thirteen years to complete and cost US\$ 3 billion. Due to the resulting breakthroughs, if it were started from scratch today it would cost US\$ 25,000 and take a week.

The chart above (Wetterstrand, 2011) illustrates the rapidly falling cost of sequencing DNA (generating the raw genetic data). Within a few years sequencing an entire genome will cost just US\$ 5,000 and be completed in a single day.

Another significant development has been born out from the study of ancient DNA; the extraction and purification of the tiny amounts of degraded DNA remaining in samples that are hundreds to tens of thousands of years old.



In 2004 Professor Andrew Lowe and his team successfully extracted DNA from timbers of the Mary Rose – King Henry VIII's Flagship which sank in 1545 and was salvaged in 1982 (Speirs *et al.* 2009)

Processed timber products such as decking, flooring and furniture are subjected to drying, engineering and treatment processes that degrade the DNA present in the wood, just as in ancient samples. As well as lowering the concentration of DNA, degradation causes the DNA to fragment into tiny pieces, making it much harder to isolate and analyse the genetic markers useful for identification purposes.

Application and adaptation of ancient DNA extraction techniques now make it possible to extract sufficient quantities of DNA from processed wood. Combined with refinements in genetic marker selection techniques (i.e. identifying markers small and robust enough to work with fragmented DNA template) it is now possible to analyse the fragmented DNA and answer critical questions about the origin and species of the wood. The time has come when *nature's barcode* can be used to manage and protect the world's forests.

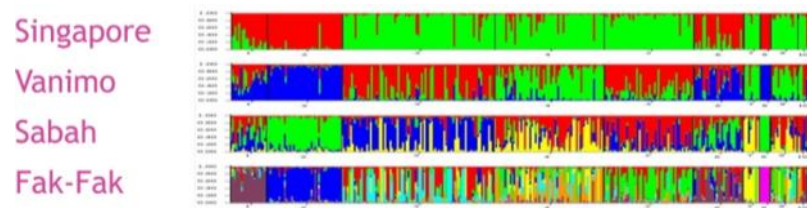
Nature's barcode

Tree DNA can be considered *nature's barcode* – a unique property inherent in trees and still present in solid wood timber products that can now be reliably and affordably read.

Figure 2 shows computer generated images of a portion of the genome of Merbau (*Intsia spp.*) from different locations of its natural range. The colouring highlights the differences between genetic marker frequencies between samples despite the fact they were taken from the same species. Similar approaches are taken to differentiate between different species, or even between individual trees.

These outputs can be utilised in much the same way as the digital black and white striped barcodes on supermarket products are used to identify products.

Figure 2
Visual representations of different populations of Merbau (*Intsia spp.*) in South East Asia (DoubleHelix 2010)



When placed in the context of international initiatives to promote sustainable trade and forest conservation, DNA technology is able to answer many critical questions that support their effective implementation and enforcement. Table 1 overleaf summarises the different scientific approaches and their relevant real world applications.

Table 1
Different scientific approaches and the questions they can answer

Scientific approach	Description	Examples of use	Page number
Population genetics	Study of genetic variation of a species across distance based on four evolutionary processes: natural selection, genetic drift, mutation and gene flow.	<ul style="list-style-type: none"> • Was this timber harvested from the declared country or region? • Does this timber come from a conservation area? • Does this timber come from natural forest or a plantation? 	11
DNA fingerprinting	A way of identifying a specific individual of the same species. Most commonly used to identify paternity and for identifying criminals.	<ul style="list-style-type: none"> • Does this product come from this log or stump (is the chain-of-custody intact) ? • Has this log or lot been swapped with other trees of the same species (log laundering) ? 	18
DNA barcoding	A taxonomic method that studies a relatively short portion of DNA to identify it as belonging to a particular species.	<ul style="list-style-type: none"> • Is this timber of the declared species? • Is this timber a CITES listed species or not? 	21

Although the scientific approaches vary, all require and will benefit from a greater body of genetic data. Research to develop genetic markers for population analysis will also generate other markers that might be suitable for DNA fingerprinting or barcoding for instance. An investment that builds capability in one area will have a positive impact across the board facilitating the use of DNA as a standard quality control tool for industry, and making enforcement of legislation easy and affordable.

An open source approach

Assembling a global genetic database for trees is a collaborative process that will build international capacity and drive adoption by business and government agencies.

We believe that an open source model is best suited to generating the large amounts of genetic data required to enable identification of origin and species for a wide range of tree species. It follows that data produced through this process should be placed in the public domain so that:

- Testing is transparent and can be verified by any interested party.
- Any organisation can develop services against the genetic data.
- Service providers compete on price and accuracy, rather than through ownership of proprietary data.

Competition in an open source marketplace revolves around developing extraction and genotyping methodologies that are cheap and reliable – both of which help build the market. It also facilitates government, industry and NGO participation in defining the nature of the services required.

An open source model also encourages capacity building as projects will involve national research bodies working alongside international centres of excellence. This creates not just a local scientific capacity but also exposure to the concept of *genetic sovereignty* for countries, and applications for conservation, biodiversity management and forest genetic resource governance (see Box 1 - The Nagoya Protocol).

Box 1 - The Nagoya Protocol

Adopted in October 2010, the Nagoya Protocol is a supplementary agreement to the Convention on Biological Diversity (CBD). It provides a transparent legal framework for the effective implementation of one of the three objectives of the CBD: the fair and equitable sharing of benefits arising out of the utilisation of genetic resources. It is therefore highly relevant to the context of creating national and international genetic databases on forests.

The Nagoya Protocol sets out core obligations for signatory countries to take measures in relation to access to genetic resources, benefit-sharing and compliance. Its adoption will have an impact on the way forest genetic data is shared and utilised.

For more information see www.cbd.int/abs/

Aside from new institutions assigned or set up as part of the Nagoya Protocol, a number of organisations might be suitable to fulfil the role of storing and providing access to genetic information:

Bioversity International (www.biodiversityinternational.org)

Bioversity's aim is to encourage and support research aimed at enhancing the sustainable use and conservation of agricultural biodiversity to the world's most vulnerable communities. Forestry is included under its remit.

Headquartered in Rome, Bioversity has around 350 staff working from 18 locations around the world and on-going work with partners in over 100 countries worldwide including close ties with UN agencies such as FAO, IFAD and WFP. It is one of 15 centres supported by the Consultative Group on International Agricultural Research.

Bioversity is establishing a database managed from a regional centre in Kuala Lumpur that can be used as a repository for genetic datasets for numerous species. This facility can act as an appropriate international caretaker for forest geographic datasets, establishing standards, ensuring data integrity and ensuring transparency and access. In March 2011, the organisation started recruiting for a new position of "Scientific Coordinator for the Identification of Tree Species and Geographic Origin."

Open Government

It is likely that national governments, whether they are signatories to the Nagoya Protocol or not, will hold copies of genetic data that pertain to their countries.

Placing this data in the public domain furthers the movement of "Government 2.0" such that all stakeholders would have access to data that helps to protect their rights and resources. In the future, indigenous peoples representatives or NGOs may access their genetic forest data as part of land rights arbitration processes for example.

Box 2 - An example of how genetic data might be held

The implementation of a large-scale project in South America would require the input and participation of local forestry research institutes in those countries where geographic mapping is taking place.

Whilst DNA extraction and analysis may take place in overseas laboratories with advanced facilities, the resulting genetic data would reside in the national genetic libraries of each participating country, with a consolidated master dataset held by a neutral international institution such as Bioversity. Key trading partners and importing countries may also hold a copy of the data so as to enable enforcement agencies to rapidly verify timber imports.

Population genetics / Genographic mapping

Imagine a world where every tree has a genetic postal code.

A forest *genographic map* shows the distribution of genetic types (genotypes) for individual trees or populations of trees across a landscape. Such a map reflects the ecological and evolutionary processes (mutation, gene flow and genetic drift) operating across tree species that have built up unique genetic variation in individuals and populations over time across the landscape charted. Several genographic mapping projects have already been achieved on a small scale (see p13), proving that it is possible to answer critical questions such as “what region or country did this timber come from?”

High-resolution maps that place the origin of a tree to within a few kilometres are feasible now, enabling differentiation between legally harvesting concessions and neighbouring conservation areas and providing unprecedented insight into the biodiversity of the forest area.

With a concerted effort by parties with common goals across the world, every tree in a forest could have its own genetic barcode sequenced, providing unprecedented levels of data to be used for a multitude of academic, conservation and commercial applications.

Applications

Independent verification of trade documentation

Comparing the DNA extracted from wood products against genographic maps will enable buyers and enforcement agencies to verify the provenance of wood products and validate declarations of the country of harvest, as required under the United States Lacey Act. Detection of false declarations at *genetic checkpoints* would be the primary means to identify shipments of illegally harvested timber. Table 2 overleaf describes a number of other situations where genetic checkpoints play an effective role to support industry and government initiatives.

Enhanced value of forest carbon/REDD projects

An emerging application of genographic mapping is for conservation schemes – especially forest carbon and REDD projects. A *genetic inventory* of commercially viable and “at-risk” forest assets is created with a sampling resolution that can determine origin down to tens of kilometres. This is a significant deterrent to illegal activity including document fraud, illegal harvesting and encroachment on project areas and enables action against illegal activity by retrospectively testing logs and timber products suspected of being removed from project areas. It also increases the value of the project by creating a base-line of genetic data from which to measure changes in biodiversity.

Of the new voluntary carbon market initiatives like the Climate, Community and Biodiversity Project Design Standards (CCB) and the Voluntary Carbon Standard (VCS), the Global Conservation Standard (www.conservationstandard.org) has been the first to incorporate genetic inventory.

Landscape restoration

A good understanding of the genetic diversity of a natural forest can be of assistance in planning or designing landscapes for reforestation, recovery from a natural disaster or future adaptation to climate change. The value of the genetic forest information can extend over several generations and future uses that are not currently envisioned may be developed in years to come.

Table 2
Genetic checkpoint applications

Application	Description / examples
Pre-purchase due diligence	<p>Major buyers can test the provenance of logs or sawn timber before buying contracts are signed.</p> <p>In one recent example a European buyer was offered logs that were claimed to be from a plantation. Unfortunately provenance paperwork was poor. The buyer evaluated the timber to be of high risk due to the prevalence of smuggling from neighbouring Myanmar. The buyer is considering the use of DNA testing to verify the log trader's claims and fulfil due diligence obligations despite the lack of documentation.</p>
Raw material testing	<p>In more complex timber product supply chains like pulp and paper, plywood and veneers, checkpoints can be set up to control the origin and species of raw material inputs. This is especially critical for high volume industries that source raw materials from a variety of sources.</p>
Wood product quality control	<p>Buyers' contracts already contain quality control clauses that allow for sampling and factory inspections during the manufacturing process. A similar provision can be introduced to allow random DNA testing of samples alongside declarations of origin and species, preventing species substitution, laundering of illegal timber into the legitimate supply chain and certification fraud without disrupting supply.</p> <p>To encourage uptake, there is an opportunity to fund genographic maps for at-risk species in regions where either fraud is prevalent or management processes are weak.</p>
Customs control at point of import	<p>Both importers and enforcement officials can now begin to consider testing at borders. The Lacey Act requires a declaration of the country of harvest and it is reasonable to envisage random selection and DNA testing of wood imports, matched against an APHIS tree database or one held at Bioversity International.</p>

Progress to date

Merbau (*Intsia spp.*)

DoubleHelix has developed a genographic map of Merbau (*Intsia spp.*) as part of a pilot project funded by the Singapore Government. A genographic map was built consisting of five different forestry concessions in Indonesia totalling nearly 1 million hectares, as well as outgroup populations from Sabah (Malaysia), Singapore and Papua New Guinea.

Blind tests demonstrated the ability to clearly differentiate wood samples taken from the different countries (Singapore, Indonesia, Malaysia, Papua New Guinea) at a confidence of over 96%. It was also possible to differentiate between samples taken from the five Indonesian concessions, but at a lower level of confidence (>70%).

Other species

Table 3 (reproduced from Lowe & Cross 2011) presents other studies of broad-scale genetic differentiation that may be used to verify timber origin. The number of studies and sources illustrates the importance of international collaboration to produce genetic datasets of sufficient number, scale and resolution.

Table 3
A selection of species subjected to large-scale genographic mapping

Species	Geographic range	References
<i>Swietenia macrophylla</i>	Central America, southern Brazil	Gillies <i>et al.</i> 1999 Höltken <i>et al.</i> 2011 Novick <i>et al.</i> 2003 Lemes <i>et al.</i> 2003, 2010
<i>Cedrela odorata</i>	Central America (Costa Rica)	Cavers <i>et al.</i> 2003, 2004
<i>Ceiba pentandra</i>	Neotropics and Africa	Dick <i>et al.</i> 2007
<i>Vouacapoua americana</i>	French Guyana and Amazon Basin	Dutech <i>et al.</i> 2000, 2003, 2004
<i>Carapa guianensis</i>	Amazon basin	Cloutier <i>et al.</i> 2005
<i>Pterocarpus officinalis</i>	Caribbean basin	Rivera-Ocasio <i>et al.</i> 2002
<i>Vochysia ferruginea</i>	Costa Rica	Cavers <i>et al.</i> 2005
<i>Lonchocarpus costariensis</i>	Guanacaste, Costa Rica	Navarro <i>et al.</i> 2005
<i>Irvingia wombulu/l. gabonensis</i>	Nigeria, Cameroon and Gabon	Lowe <i>et al.</i> 2000, 2010a
<i>Aucoumea klaineana</i>	Gabon	Muloko-Ntoutoume <i>et al.</i> 2000

Planned projects

Large scale genographic mapping of African Congo Basin forests

This project aims to develop and implement a species identification and timber tracking system for three commercially traded timber species – Iroko (*Milicia excelsa*), Sapelli (*Entandrophragma cylindricum*) and Ayous (*Triplochiton scleroxylon*) - across seven countries across the African Congo Basin (see figure 3). It will apply a combination of genographic mapping, DNA fingerprinting and chemical isotope approaches.

The International Timber Trade Organisation (ITTO) has approved funding by the German Government and is expected to formally sign off in December 2011 with work underway from January 2012. It will bring together the expertise and resources of a number of organisations including the Institute of Forest Genetics at the Johann Heinrich von Thünen Institute in Germany (the project coordinator), the Université Libre de Bruxelles (Belgium), the University of Adelaide (Australia), NERC Centre for Ecology and Hydrology (UK) and DoubleHelix (Singapore).

The Forest Research Institutes of Ghana and Kenya as well as the Institute de Recherche en Ecologie Tropicale in Gabon are expected to participate in the project. A number of local partners in the African countries will assist with sampling for the reference data bases and for the blind tests.

The project is planned to run over three years and has a confirmed budget of US\$ 1.7 million with a total estimated requirement of US\$ 3 million.

Figure 3
Scope of ITTO Congo Basin project



The seven African countries involved in the project are shown in yellow. Red stars indicate the location of the three genetic reference laboratories in Kumasi (Ghana), Libreville (Gabon) and Nairobi (Kenya).

It is also hoped to develop species barcodes for up to 20 African species, some of them CITES protected and those that can be easily confused with them.

DNA fingerprinting approaches developed by DoubleHelix (see p18) will also be applied to a number of supply chains exporting to Europe.

Future opportunities

Similar genographic mapping projects can be of value to other regions or countries that suffer high levels of illegal logging and income loss. In all the following examples, work would be conducted in collaboration with the appropriate local scientific institutions and experts.

Russian Far-East – various species

Illegal logging is a significant problem across Russia and is believed to result in a direct loss to the Russian economy of US\$ 1-3 billion annually (Crowley 2005). Following the imposition of strict restrictions on the logging of Chinese forest in 1998, illegal logging has increased in the Russian Far East such that the volume of illegal timber exported from the region is now believed to exceed the level of official export (Kramme 2011).

Utilising the suite of DNA approaches, multiple objectives could be achieved:

1. Tackle illegal logging and smuggling of timber from Russia to China and protect Russian forests.
2. Detect illegal logging and trade of CITES listed species such as Korean Pine and Amur Cork.
3. Protect the environment of the endangered Amur Tiger.
4. Deter attempts to sell products manufactured in China from Russian timber and sold in the United States or Europe as American timber.

With a budget of US\$ 750,000 it is feasible to create genographic maps of up to 6 key species in the region and address these issues.

South America – Spanish Cedar (*Cedrela spp.*)

As well as being one of Latin America's most prized and exploited timber species, Spanish Cedar is notable in that it is the subject of multiple regulations to control its harvest and trade.

Of the three commercially traded sub-species (*Cedrela odorata*, *fissilis* and *lilloi*), only *odorata* has been listed on CITES Appendix III (by Peru and Colombia). The drawbacks of an Appendix III listing are two-fold. Firstly, it encourages smuggling of *odorata* to neighbouring states where it is not CITES listed and therefore not subject to export restrictions (see p16). Secondly it encourages the mis-declaration of *odorata* as *fissilis* or *lilloi*, both of which are indistinguishable from *odorata* to the untrained eye and again not subject to export restrictions.

The unintended impact of the Appendix III listing in 2002 has been an estimated five-fold increase in export volumes of Spanish Cedar, of which up to 70% could be of illegal origin (INRENA 2008).

In addition, the US-PERU-FTA includes an annex on Forest Sector Governance designed to address the illegal timber trade and enhance the protection of CITES listed species. Clause 3(h)(ii) of the Annex on Forest Sector Governance requires Peru to “Develop systems to verify the legal origin and chain-of-custody of CITES-listed tree species and develop systems...to reliably track specimens from harvest through transport, processing and export.”

An estimated budget of US\$ 850,000 would be required to create useful genographic maps of Spanish Cedar to tackle log smuggling associated with circumventing CITES controls and support the objectives of the US-PERU FTA.

- South East Asia – Teak (*Tectona grandis*)

Teak is a valuable hardwood timber species, traded globally and used for many different applications. At present the species is extracted from both natural forests and plantation stocks, but demand outstrips supply. Harvesting is banned or heavily restricted in the countries that comprise its natural range which includes India, Myanmar, Thailand, Laos and Indonesia (Verhaegen *et al.* 2005), though enforcement of logging restrictions is insufficient. In order to prevent trading of illegally logged timber and to promote the sustainable harvest of natural populations it is necessary to be able to verify the source of teak timber and its products.

A budget of approximately US\$ 1 million would be sufficient to develop genographic maps of the natural ranges of Teak and enable verification of country of harvest.

DNA fingerprinting

DNA fingerprinting can differentiate between individual trees that were harvested from the same concession – even neighbouring stumps. It is based upon methods already used in criminal forensics and paternity testing.

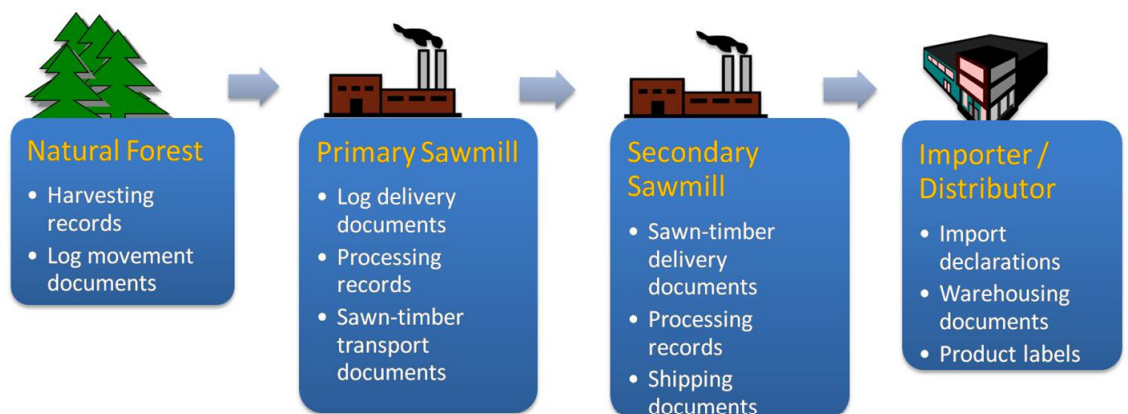
Applications

There are well recognised systems to assess and certify sustainable and legal timber supply chains that have successfully established global brands. This has been beneficial to the development of sustainable forest practices and growing demand for certified timber products. Joining the established market are emerging, often national, timber legality systems in producer countries that are developed as part of EU-FLEGT and other capacity building programmes.

All certification and legality verification systems must have a mechanism to track timber from the legitimate forest source through to export. Such tracking or *chain-of-custody* systems are designed to exclude timber from other unknown or illegal sources, as well as enable independent monitoring to provide assurance to all interested parties that the system is working as planned and maintain its credibility (FLEGT 2007).

Figure 4

A typical document based tracking system is vulnerable to fraud



Like any successful brand, a market for imitation and fraud has followed, whereby illegal timber is introduced into certified supply chains (laundering) and passed off to the market as certified product. This is due to the fact that paper-based wood tracking systems used by most timber certification schemes are highly vulnerable to document fraud (see figure 4).

The most obvious application of DNA fingerprinting of timber is to provide a truly independent, scientific verification of the tracking system. Introduction of DNA testing into these systems is not only an effective measure to deter document fraud, cutting off log laundering channels, but also to protect the certification brands, lower cost, facilitate uptake and increase transparency.

The process is simple to implement. Wood samples are taken from trees prior to harvest, during the forest inventory process. These samples are stored so that they can be tested and analysed at a later date. During harvesting and processing, samples are taken from the same trees and logs, according to the tracking system documentation. This second set of samples is physically matched with the samples taken during the inventory. If the tracking documentation is correct, then the paired samples should come from the same trees. DNA fingerprinting will scientifically verify that they are from the same trees by comparing their individual genetic profiles. If the genetic profiles do not match, then a breakdown in the system, accidental or deliberate, has occurred and the system auditors can take targeted, direct action to rectify the problem.

Though basic paper-based systems are still needed to match samples back to their source logs, the incentive to abuse these systems on the part of any company or individual is removed, since it will be exposed by DNA fingerprinting. The targeted nature of DNA fingerprinting also allows auditors to reduce the intensity and frequency of regular physical audits. Since chain-of-custody audits make up a significant proportion of certification costs, it follows that a reduction in auditing time and effort all along the certified supply chain will reduce the overall cost of timber certification.

Not to be underestimated is the potential of DNA to enhance the credibility and trust associated with a brand. Popular knowledge of DNA technology applied to criminal forensics means that consumers and buyers recognise the capabilities of DNA testing, increasing trust in and awareness of associated certification schemes whilst at the same time deterring illegal timber laundering through DNA verified supply chains.

Progress to date

In 2009, the International Tropical Timber Organisation (ITTO) supported a timber tracking project conducted by DoubleHelix to evaluate the scientific viability of DNA timber tracking integrated into an existing document based Chain-of-Custody system. The project was conducted on a Merbau (*Intsia* spp.) supply chain, with logs harvested in Papua, Indonesia, then transported to a mill in Java for processing into flooring and decking. The results demonstrate reliable and accurate differentiation between individual trees/logs and validate the transportation documentation from concession to mill.

The detailed results from this ITTO funded project have been published in the peer reviewed scientific journal *Silvae Genetica* (Lowe *et al.* 2010).

Planned and existing programmes

Similar projects are planned for implementation as part of the ITTO African Congo Basin project (see p14) and as part of a project to support the development of an advanced timber tracking system in Ghana.

Future opportunities

The ITTO timber tracking project provides pointers for further development. In order to achieve implementation on a small scale, the project was conducted on specific batches of timber, and on a limited portion of the supply chain (from the log pond to delivery at the primary sawmill).

A next step would be to apply this methodology to an area-based certification system such as the Forest Stewardship Council (FSC) or Indonesia's newly launched timber legality system (SVLK), in collaboration with a reputable Certification Body. Sampling would also extend further down the supply chain through processing and on to finished product, with a focus on control points most at risk from log or lot swapping.

Such an approach could be integrated into the Timber Legality Assurance Systems (TLAS) being developed by countries participating in the EU-FLEGT Voluntary Partnership Agreements. It is likely that TLAS will involve improvements and additions to existing government systems and DNA could be used either as part of a standard in-country verification process or, when combined with *genetic checkpoints* (see p11), jointly utilised by the EU and source country governments as an enforcement and verification tool.

The estimated running cost of a DNA verification system for a certified supply chain would be as little as US\$ 18,000 per year. Initial research and investment would be required to identify genetic markers suitable for DNA fingerprinting new timber species, but this investment would be spread across all trees of the same species worldwide.

DNA barcoding

Simple and irrefutable species identification for CITES control and other illegal activities in the timber trade.

Currently, anatomical characteristics (such as microscopic analysis to examine the cell structure of wood samples) are used to identify trees down to genus or family level. Species identification is often not possible, particularly for species with closely related sub-species such as Mahogany. Furthermore, worldwide capacity of wood anatomical expertise is limited, utilised only in rare cases.

DNA barcoding enables accurate verification of species at any level and provides ample testing capacity to make regular testing of suspicious shipments an affordable reality. The impact – practical tools to protect those species most at risk from over-exploitation and extinction.

Applications

CITES listed trees suffer from illegal exploitation and can be difficult to identify once converted to timber. CITES Appendix I, II and III listed species pose different detection problems. CITES I and II listed species can be falsely declared as similar looking, but non-listed species. In the case of Appendix III species, where trade controls are only applied in certain countries, timber can be smuggled across borders to where the species is not CITES listed.

Despite its inclusion on Appendix I of CITES, Brazilian rosewood (*Dalbergia nigra*) continues to be illegally logged and traded internationally. In 2009, police in Rotterdam seized 249 sheets of Brazilian rosewood. It can be listed as *Dalbergia cearensis*, a close relative with similar anatomical characteristics, which is not CITES listed.

Three species of Mahogany (*Swietenia humilis*, *macrophylla* and *mahagoni*) are listed on Appendix II. In order to avoid CITES export declaration controls these species are again declared as non-CITES listed species. Following recent confiscation of a suspected Mahogany shipment by police in Germany, wood anatomists were unable to differentiate between the Mahogany species whereas a genetic test was able to verify and prove beyond question the species of the timber.

Progress to date

The International Barcode of Life (iBOL) is the largest biodiversity genomics initiative ever undertaken. Hundreds of biodiversity scientists, genomics specialists, technologists and ethicists from 25 nations are working together to construct a DNA barcode reference library that will be the foundation for a DNA-based identification system for all multi-cellular life.

At the same time the Consortium for the Barcode of Life (CBOL) is working to publicise the benefits and opportunities arising from DNA barcoding and seeks to encourage collaboration and the development of international standards and protocols.

Barcoding projects have four components (CBOL 2010):

1. The specimens
Natural history museums and herbaria will be key resources to collect identified specimens, but it will also be necessary to collect additional samples of specimens from the field.
2. The laboratory analysis
Laboratory protocols developed by iBOL and CBOL can be followed to obtain DNA barcode sequences from the specimens. The best equipped molecular biology labs can produce a DNA barcode sequence in a few hours. The data is then placed in a database for subsequent analysis.
3. The database
One of the most important components of the Barcode Initiative is the construction of a public reference library of species identifiers. There are currently two main barcode databases that fill this role:
 - i. The International Nucleotide Sequence Database Collaborative is a partnership between GenBank in the United States, the Nucleotide Sequence Database of the European Molecular Biology Lab in Europe, and the DNA Data Bank of Japan. They have agreed to CBOL's data standards for barcode records.
 - ii. The Barcode of Life Database (BOLD) is maintained by the University of Guelph in Ontario, Canada. It offers researchers a way to collect, manage, and analyze DNA barcode data.
4. The data analysis
Specimens are identified by finding the closest matching reference record in the database. The Barcode of Life Data Portal has been developed to allow researchers new and more flexible ways to store, manage, analyze and display their barcode data.

DoubleHelix, in conjunction with a team at the Smithsonian Institute, is leading the working party tasked with generating DNA barcodes for what is thought to be up to 150,000 tree species on the planet. The initiative is called the Tree Barcode of Life or TreeBOL. The tree barcode data will be stored at the BOLD facility. So far 10,000 sequences representing approximately 5,000 species have been submitted for data analysis.

Future opportunities

DoubleHelix plans to integrate these DNA barcoding efforts with current plant taxonomic and systematic research underway internationally, as well as begin new projects in plant groups that have received less attention.

Data from the barcoding project will be mutually beneficial to all plant researchers involved. There will be many opportunities for additional analyses from the data generated by the TreeBOL project, which can lead to scientific research at many scales. This can include population genetic processes up to phylogenies of specific genera and families, and even across families. The additional studies coming out of the barcoding project will enhance scientific understanding of plant evolution and will improve the methodologies used for species identification.

We estimate that US\$ 7 million would sufficiently fund the entire barcoding of all living tree species in the world, which is composed of US\$4 million for lab work, US\$2 million for collection and curation and US\$1 million for meetings and project management over a five year period.

Conclusion

The question is not if applied genetics has a role in conservation and timber supply chain management, but when the tipping point leading to routine genetic testing will be reached.

It is clear that genetics has an important role to play in the management and protection of forest resources, with a growing body of evidence that demonstrates how applied genetics can support the sustainable trade of timber, reduce price incentives for illegal activity and support conservation efforts.

There is more than enough scientific evidence to demonstrate that genetic methods are ready to supplement or replace non-genetic methods and in some cases provide tests for which no other independent scientific technique can verify. The advancement of genetics technologies means that large-scale genographic mapping can be done cheaply, routinely, quickly and with a statistical certainty than can be used in a court of law (Lowe & Cross 2011). The framework for international collaboration is developing, guided by the Nagoya Protocol and with new institutions taking on responsibility for standard setting and best practice.

Whilst the continuing work and increasing numbers of international, large-scale projects across all these areas will increase awareness and encourage adoption, more effort is required. Barcoding tree species as part of International Barcode of Life project requires combined action from a Foundation with a remit for long term investment in environmental conservancy and farsighted private companies with a commitment to Corporate Social Responsibility. Building of genographic maps is more likely to fall under the purview of national governments, international bodies concerned with governance and environmental issues and Foundations. Application of DNA to chain-of-custody systems will require an understanding and demand from buyers as well as an interest from certification systems to improve their existing practices.

The question now is how quickly we can marshal the resources to increase capacity of forest genetics and bring about profound improvements in the way forest resources are managed, protected and sustained.

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