PROJECT COMPLETION REPORT TO THE
INTERNATIONAL TROPICAL TIMBER ORGANISATION (ITTO)
Submitted by Double Helix Tracking Technologies Pte Ltd

Pilot Implementation of a DNA traceability system for *Pericopsis elata* in forest concessions and sawmills in Cameroon and Congo.

PERIOD OF REPORT	01 March 2014 to 31 March 2016
PROJECT NUMBER	PP-A/39-162A
EXECUTING/IMPLEMENTING AGENCY	Ministry of Forest Economy and Sustainable Development, Congo Ministry of Forestry and Wildlife (MINFOF), Cameroon
COLLABORATING AGENCIES	Double Helix Tracking Technologies, Singapore Association of Timber and Forest Industries (ATFI), Cameroon
DURATION	24 months
START DATE	April 2014
PROJECT BUDGET	US$ 303 500
Project coordinator	Double Helix Tracking Technologies Pte Ltd 105 Cecil Street, #22-00 The Octagon Singapore 069534
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EXECUTIVE SUMMARY

This project is the latest in a series supported by the ITTO focusing on the development of DNA techniques for timber traceability. It provides a real study of impact on forest and sawmill operations and shows that the likely running costs of such a system is a small percentage of product value. The results of this project, taken together with the *Prunus africana* project undertaken concurrently, provide a useful comparison of different approaches to DNA tracking from which to plan future activities at a larger scale.

This pilot project was designed to demonstrate that *Pericopsis elata* logs and sawn timber can be traced back to specific stumps located in forest concessions using DNA techniques. The intention is to detect and deter substitution or addition of *Pericopsis* logs between forest concessions and sawmill or point of export, increase supply chain transparency and bring greater confidence in the control of harvest volumes.

All project outputs and activities were successfully carried out. The project completion was delayed a total of 12 months due to difficulties in sample collection, changes in harvest and timber processing schedules, and the slow issuance of CITES export permits for scientific samples.

Tangible outputs from the project include a suite of 45 genetic markers that can be used to assign samples back to individual trees with a high degree of confidence. A further 313 genetic markers are available for future analysis to determine suitability for population assignment. A comprehensive DNA verification system has also been designed that allows for routine verification of upstream chain of custody between forest and sawmill, and between sawn timber of log input.

Key lessons learnt were in relation to improving field sampling practices to avoid errors in collection and sample recording. The practical application test did reveal breakdowns in the existing chain of custody system, especially when trying to link sawn timber back to claimed logs cut. Having trialed two DNA methodologies (individual and population assignment) across the two projects, it is now also clear that population assignment is a more suitable technique to support the CITES permit process. Individual assignment is appropriate where national governments may wish to strengthen timber chain of custody procedures or the monitoring of sustainable harvest plans.

Irrespective of the methodology used, before a DNA-based system is rolled out more widely, it is strongly recommended that a review of the CITES permit process is undertaken to increase the speed of approval for scientific samples, otherwise routine testing and reporting of results will be too slow to inform decision-making.

With a focus on population assignment, genetic reference data will be more easily established for other forest concessions exploiting *Pericopsis elata.* This will enable assignment of samples back to forest and verify claims of origin in the same way as has been demonstrated for *Prunus africana.*

1 PROJECT IDENTIFICATION

1.1 Context

This project was carried out at the request of the ITTO to support the ITTO - CITES program "CITES implementation for tree species and trade/market transparency (TMT). Specifically, this proposal aims to support the ITTO – CITES Component expected output (e) "*Improved capacity for the installation of modern, effective information and tracking systems*" as well as the TMT component. The activity is consistent with both Congo's Forest Law and Cameroon's Forest and Environment Sectorial Program (FESP) developed in 2003 (MINEF 2003) and revised in 2010.

The International Tropical Timber Organisation (ITTO) has been an active proponent of the development of DNA techniques for timber traceability. Such systems were first introduced by Double Helix Tracking Technologies Pte Ltd (DoubleHelix) in the Indonesian forestry sector in 2007, verifying Chain-of-Custody documentation of merbau (*intsia* spp.), processed for export to Australia, New Zealand and Europe.

The ITTO supported the implementation of this system in another Indonesian supply chain in 2010 (ITTO Activity No.: PP-A/43-194 - Promote Trade in Tropical Timber and Tropical Timber Products from Sustainably Managed and Legally Harvested Sources). Project results were published in the peer-reviewed scientific journal Silvae Genetica[1] and a recent ITTO-CITES publication[2].

Other ITTO funded DNA projects have been carried out in the Congo Basin region. PD 620/11 Development and Implementation of a Species Identification and Timber Tracking System in Africa with DNA Fingerprints and Stable Isotopes involved genetic and isotope mapping of multiple tree species across seven African countries.

In parallel with this project, DoubleHelix has implemented a pilot project for *Prunus africana*. The findings and conclusions of this and the *Prunus* project should be considered together in order to determine the most appropriate next steps for introducing DNA traceability more widely.

1.2 Origin and problem

Reliable and effective timber tracking systems are an essential component of the ITTO-CITES programme. Great efforts have been made to implement sustainable harvest plans and conduct non-detriment findings required by the programme to establish legal export quotas.

Attempts to circumvent CITES controls have the potential to undermine confidence in the programme however. In 2013, a shipment from a concession in north Congo was seized in Belgium, since documentation claimed that it came from a different concession than the one participating in the ITTO-CITES programme. A similar problem was observed in Cameroon, with *Pericopsis elata* timber exported to Belgium but claimed to be harvested from areas out of the ITTO-CITES programme. These are two examples where no attempt has even been made to disguise the illegal origin. Indeed, it is currently impossible to determine the level of false claims of origin.

Through the successful implementation and roll out of this project, it will be possible to build a picture of levels of attempted substitution and inaccurate claims. Better monitoring of controlled supply chains and associated transparency will lead to an increase in confidence. It is hoped that such improvements will eventually deter substitution and allow for an increase in export quotas based on hard scientific data and confidence that CITES controls are effective.

2 PROJECT OBJECTIVES AND IMPLEMENTATION STRATEGY

2.1 Objectives and adjustments

The original project objective was to demonstrate that *Pericopsis elata* logs and sawn timber can be traced back to specific stumps from a controlled concession (one that has undergone a NDF and is implementing an authorized harvest plan).

Routine application of this approach would enable random verification of traceability documentation to detect log substitution and fraud to a high level of confidence, allowing for timely corrective actions to be implemented.

There were no adjustments to the scope of work during the project, although the project duration was extended due to delays in sampling and sample shipment.

Additional budget was requested at the end of the project to cover time and expenses associated with attending the closing workshops for both *Pericopsis elata* and *Prunus africana*.

2.2 Implementation strategy and adjustments

The implementation strategy consisted of:

Preparation and development of DNA reference data suitable for individual matching

- Preliminary work to confirm the ability to extract DNA from *Pericopsis elata* sawn timber. If unsuccessful, this would have been a severely limiting factor to successful project utility and completion.

- In parallel with the above, a training session was held in Kribi, Cameroon, with representative from Congo travelling to Cameroon. The training consisted of procedures for sample collection, recording, storage and shipment. The training was combined with the opening workshop, with practical field trips conducted in the days following the workshop.

- After training, sampling from standing trees was conducted in different locations in the south of Cameroon and northern Congo.

- These samples were sent to the University of Adelaide for analysis and development of genetic markers (a process called ‘genotyping’) suitable for individual matching and identification. This is a similar approach to that used in human criminal forensics to match evidence samples to suspect DNA.

Validation of genetic markers / testing of supply chain samples

- Industry partners were identified that were willing to allow access to forest concessions, log yards and sawmills for sampling of logs and sawn timber.

- Based on existing chain of custody documentation, samples were collected from the same logs / timber at various points along the supply chain. These samples were recorded, packed and sent in two separate batches to the University of Adelaide for analysis.

- On arrival at the University of Adelaide, the two sets of samples would be physically matched together and prepared for testing.

DNA testing and analysis

- After DNA was extracted from the wood samples, it was analysed and the genetic markers compared. If the DNA test confirms a genetic match between the paired samples, the Chain-of-Custody is proven intact. Alternatively, a genetic mis-match indicates that the paired samples come from different trees than claimed. This is evidence of at best, a breakdown in the chain of custody, and at worst an indicator of log substitution from non-controlled areas at some point between where the two sets of samples were taken.

- Statistical analysis shows the level of confidence associated with the DNA test results.

2.3 Risks and assumptions

No major risks were identified during the project proposal. The initial risk of failure to extract DNA from sawn timber was minimized through the first activity of the project: trial DNA extraction, which was successfully completed.

Assumptions were made that there would be no problems associated with sampling or transport of samples to the laboratory in Australia, or with harvest and processing chain of custody procedures that would allow for document matching of timber back to individual trees. These were areas that proved problematic during the execution of the project.

3 PROJECT PERFORMANCE

3.1 Achievement of project outputs and activities

What follows is a comparison between the project outputs and activities as originally devised and the final outcomes:

Output 1.1: Development of genetic markers for *P. elata* suitable for DNA fingerprinting (differentiation of wood between individual trees of the same species).

Activity 1.1.1 Trial DNA extraction from wood samples

Project document description	Initial tests will be performed on *P. elata* wood samples to confirm that DNA of sufficient quality and quantity can be extracted from the wood samples on a routine basis.
Changes to activity	None
Realized activity	Completed May 2014. A preliminary test to verify that DNA could be extracted from *P. elata* timber was successfully completed. Five samples were submitted to the laboratory for testing and DNA of sufficient quantity and quality was extracted from all five. The results of this DNA extraction test are presented in **Appendix A**.

Activity 1.1.2 Population sampling

Project document description	Additional samples will be collected from a number of different populations within the species range. 10 samples each from 5 different populations across Cameroon and Congo will be collected.
Changes to activity	Due to concerns over the accuracy of sample collection data, additional samples were obtained from the Thünen Institut. This was done at no additional cost.
Realized activity	Complete. 170 samples were collected across three different locations in Cameroon. 335 samples were collected across two different locations in Congo. This exceeded the sampling objectives, however a large proportion of samples were unusable for analysis due to inaccurate sampling data. As a result, a set of 13 additional *vouchered* samples with validated location data were obtained from the Thünen Institut in Germany. Whilst many samples were unusable, a large proportion of these samples will be suitable to develop population assignment markers in any extension to this project. For more detail on the sample quantities and locations, refer to **Appendix B**.

Activity 1.1.3 Genetic marker development

Project document description	The samples from the above populations will be processed in the laboratory to identify 'polymorphic' genetic markers. These are markers that can be used to differentiate between individual trees of the same species.
Changes to activity	No change
Realized activity	Complete. Using the vouchered samples, a total of 358 Single Nucleotide Polymorphisms (SNPs) were identified. 97 of these were screened and a final set of 45 SNP markers were selected for use in the final testing. In order to demonstrate that matching DNA profiles are obtained when comparing cambium and wood samples from the same tree, 15 forest samples from the Congo were split in the laboratory into paired cambium and wood sub-samples (small parts of both tissue types were collected). The genotypes obtained from each of these sub-samples were compared to their counterpart from the same tree to assess accuracy of the method. For more detail on the marker development and accuracy of the testing methodology, refer to **Appendix C**.

Output 1.2: Capacity building and training of local teams in DNA sample collection and storage.

Activity 1.2.1 Workshops for stakeholder consultation and field training

Project document description	An opening and closing workshop to inform, receive input and report back to key stakeholders involved in the ITTO-CITES project. Suggested participant organisations include CNIAF in Congo, government representatives, SIFCO, sawmill representatives, customs representatives and EU buyers of timber. The opening workshop will include a field trip to conduct training of staff in wood sampling techniques and can also be combined with the population sampling (Activity 1.1.2).
Changes to activity	The opening workshop for Cameroon and Congo was combined and held in Kribi, Cameroon.
Realized activity	In Cameroon we were able to combine the workshop and training for both *P. elata* and *Prunus africana* projects. Agendas, attendance lists and photographs from the workshops have been provided in the mid-term project report delivered December 2014. The closing workshop for Congo was scheduled for March 31st 2016 in Brazzaville, but was cancelled at short notice due to fears of local civil unrest. No further arrangements have been made to reschedule the meeting, however a planned regional workshop is expected to take place later in 2016. The Cameroon closing workshop was held on 1st April 2016 at the ANAFOR offices in Yaoundé.

Output 1.3: Implementation of DNA verification in three controlled supply chains in Cameroon and Congo from pre-harvest to point of export.

Activity 1.3.1 Verification system development

Project document description	This consists of identifying appropriate stages in the forest management, harvesting and transportation process to take samples from logs and sawn timber. Care will be taken to work with concession and the national ITTO-CITES project coordination teams to understand the field processes and design a sampling system that uses existing resources with minimum impact on forest and sawmill operations. At the end of this stage a report will be presented explaining the system design, statistical sampling strategy and resulting estimated level of confidence. (For example, “through this system we will be able to detect log substitution amounting to 1% of total volume, 99% of the time”).
Changes to activity	Testing and analysis was conducted in two supply chains – one in Cameroon and one in Congo.

Realized activity	Complete. A number of phone calls and meetings were held with members of ITTO, ANAFOR, the Ministry of Forest Economy and Sustainable Development, as well as industry representatives from the forest concessions and mills sampled during the project. See **Appendix D** for a list of stakeholder meetings and calls held during both projects. Sample matching and testing was successfully demonstrated across two supply chains and verification tests were applied at two control points along the supply chain. Having trialled and evaluated both an individual matching (*Pericopsis)* and population assignment (*Prunus*) methodologies, we have concluded that population assignment is the more suitable for CITES permit control. This conclusion was presented at the closing meetings and met with unanimous approval. For more information on the population assignment methodology, prefer to the *Prunus* project final report.

Activity 1.3.2 Field implementation

Project document description	The following basic process will be adapted according to the research and findings made during Activity 1.3.1 Verification system development. i) Wood samples are taken from the cambium (the layer underneath the bark of the tree) of *P. elata* trees either prior to harvest (during forest inventory) or during harvest. ii) A second set of cambium samples are taken from logs after harvest at an appropriate control point. Control points under consideration could be one or more of a) the forest log parc, b) village log parc, c) sawmill log yards, d) prior to loading onto trucks for transport to Douala, e) at Douala Port prior to export or f) on arrival in the EU. iii) Using CoC documentation, the cambium samples taken pre- and post-harvest are physically matched together. iv) A statistically representative number of paired cambium samples are selected and sent to the DoubleHelix laboratory for DNA testing. The number of paired samples to be tested and the frequency of testing will depend on the harvest plan and volume of timber harvested in each period as well as the preferred timescales to identify problems and implement corrective actions. DoubleHelix has previously applied **ISO 2859: Sampling procedures for inspection by attributes** to calculate the number of samples to be tested according to the level of confidence required. A similar approach will be considered for this project.
Changes to activity	No change
Realized activity	In Cameroon: • 24 cambium samples were taken from trees prior to harvest. • A further 21 cambium samples were taken from supposedly the same logs on delivery to the saw mill. • A further 23 timber samples were taken from sawn timber after cutting of the supposed same logs. In Congo: • 15 cambium samples were taken from trees prior to harvest. • A further 14 samples were collected from supposedly the same trees on delivery to the saw mill. **Appendix C – Test samples** details the type and location of all samples collected for pairing and matching.

Activity 1.3.3 Ongoing DNA testing

Project document description	The remainder of the DNA verification process is conducted in the laboratory and does not impact forest operations. After DNA is extracted from the wood samples, it is analysed and the genetic markers compared. If the DNA test confirms a genetic match between the paired samples, the Chain-of-Custody is proven intact. On the other hand, a genetic mis-match indicates that the paired samples come from different trees. This is evidence of log substitution and the Chain-of-Custody may have been broken.
Changes to activity	No change
Realized activity	Successful DNA extraction and genotyping was variable, with a higher rate of success (96%) from forest samples compared to mill samples (69%). Success is measured by the laboratory in terms of the number of markers that can be successfully amplified. This threshold was set at 24 out of the possible 45 markers. Following genotyping, the samples were submitted for paired testing. For Cameroon samples: • Cambium samples from 18 logs taken at the saw mill prior to processing were matched to samples taken from forest. 13 (72%) of samples matched. 4 (22%) samples did not match claimed samples, but did match other samples from the same forest. One (6%) did not match any sample. • 14 sawn timber samples were matched with cambium samples taken prior to processing. Of the 14 samples, three (21%) matched an individual as claimed. 11 (79%) did not match any samples successfully genotyped as part of the project. For Congo samples: • Cambium samples from 14 logs in the log yard were compared to samples taken from the standing trees prior to cutting. 11 (79%) were confirmed as a match. 3 (21%) did not match their claimed counterpart nor any other samples genotyped during the project. For further detail and charts of the test results, see **Appendix C**.

Activity 1.3.4 Statistical analysis of results

Project document description	A full analysis of the results of all the paired samples will be conducted and a final conclusion drawn on the likelihood of systematic substitution within the supply chain. The test results and statistical analysis will be provided in a report delivered regularly according to the frequency of testing selected.
Changes to activity	No change
Realized activity	If all 45 genetic markers are matched during a test, the probability of a random match (a false positive) is 1 in 390 billion. For related individuals or siblings (more likely where samples have been collected from trees in close proximity), this probability increases to 1 in 798,583. The minimum number of genetic markers that were matched in a successful pairing was 29. At this level of matching, the probability of a false negative is 1 in 7714 trees. A full probability table is presented in **Appendix C**.

3.2 Comparison of activity progress against schedule

Table 1 below shows the project implementation in comparison with the detailed workplan provided to ITTO in March 2014. The approved workplan schedule is marked in grey, with the actual implementation indicated by the hatched red cells.

Significant delays were attributable to delays in sampling in Congo, due to changes in harvest planning. This disrupted plans to collect matching samples from both forest and saw mill. In order to minimize laboratory costs, final testing of both Cameroon and Congo samples was delayed until all samples from Congo were received at the laboratory. This did not take place until late December 2015.

Table 1: Activity schedule in comparison with workplan

OUTPUTS / ACTIVITIES	RESPONSIBLE PARTY	Month																							
		Apr '14	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan '15	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan '16	Feb	Mar
Output 1.1																									
1.1.1 Trial DNA extraction	DoubleHelix																								
1.1.2 Population sampling	CNIAF, ANAFOR, DoubleHelix																								
1.1.3 DNA marker development	DoubleHelix																								
Output 1.2																									
1.2.1 Workshops and training	CNIAF, ANAFOR, DoubleHelix																								
Output 1.3																									
1.3.1 Verification system development	DoubleHelix																								
1.3.2 Field implementation	CNIAF, ANAFOR, DoubleHelix																								
1.3.3 Ongoing DNA verification	DoubleHelix																								
1.3.4 Statistical analysis	DoubleHelix																								

3.3 Project expenditure against funds input

Total project expenditure to date by DoubleHelix: USD 147,823

This figure consists of:

- USD 90,908 of direct expenses
- USD 56,915 of project coordination and reporting costs

Note: This figure does not include additional expenses to attend project closing workshops in Cameroon and Congo, for which additional budget was granted by ITTO. Also note that excess funds have been assigned to cover budget shortfall related to Prunus *project work.*

Due to the extension of project duration from 12 to 24 months, project personnel and administration fees were higher than originally planned by USD 12,815. This was offset by an exchange rate gain over the course of the project as the Australian Dollar weakened against the US dollar making laboratory fees USD 21,000 lower than budget.

For further detail on expenditure against budget see **Appendix E**.

- Total funds received from ITTO to date: USD 130,000
- Remaining funds to be released: USD 26,100

4 PROJECT OUTCOME

The overall objective as stated in the project proposal was achieved. The project demonstrated how sawn timber and log samples can be matched back to specific trees and stumps from a controlled concession using DNA fingerprinting techniques.

Furthermore, a test application of DNA fingerprinting across two supply chains identified several instances where a breakdown in document chain of custody seems to have occurred. The degree of confidence associated with the matching test was very high.

This type of testing is more suitable for chain of custody control at the upstream end of the supply chain and could be used to ensure that forest concessions adhere to sustainable harvesting plans.

When compared to the system implemented for *Prunus* supply chains (population assignment), it is clear that the population assignment is more suited to the purpose of supporting the CITES permit process. This is an important learning and outcome for the project as a whole.

4.1 Tangible outputs

- A suite of 45 SNP genetic markers validated and suitable for individual matching of *Pericopsis elata* wood and timber samples.
- A further 313 SNP markers that can be screened in future projects to identify those suitable for population assignment.
- Training and knowledge transfer to sampling teams in Cameroon and Congo.
- A system to verify chain of custody documentation between forest and sawmill, as well as sawn timber back to log input.
- Test result data on chain of custody integrity in Cameroon (both forest to sawmill and sawn timber to log input) and Congo (forest to sawmill).
- Associated random match probabilities indicating level of confidence associated with DNA matching based on the number of genetic markers utilized.
- A comparison of individual and population assignment methodologies between the *Prunus* and *Pericopsis* projects has determined that population assignment is a workable method to verify CITES permit claims of origin.

4.2 Participation of target beneficiaries

- Training of sampling teams from Cameroon and Congo on sampling techniques, sample packing, storage and logistics. Participants in the training included those from local academic institutions, universities, Government departments and industry.
- Participation of industry in sampling process at FMEs and sawmills.
- Ongoing communication with industry in Cameroon and Congo.
- Discussion with CITES authorities on potential implementation of systems in Cameroon.

4.3 Next steps

We recommend the following next steps:

- Peer review and publication of the scientific work.

- A focus on the population assignment approach for future work. We recommend evaluating the current suite of 358 SNP markers to discover those suitable for population assignment. This work can be done without additional field sampling.

- Establish a genetic reference database of all populations of *Pericopsis elata* under the ITTO-CITES programme.

- Formalisation of the procedures for sampling, testing and reporting in consultation with Government, the CITES Secretariat, industry and other stakeholders.

- The roll out of a screening programme (routine verification of CITES permits) to control risk of substitution from non-authorised harvest areas.

- Create a fast-track CITES permit issuing system for scientific samples to ensure speed of delivery of test samples to laboratories.

- Develop capacity to conduct testing within the region. As part of the ITTO Congo Basin project, basic lab facilities have been set up with varying success in Kenya, Ghana and Gabon. A proposal was submitted to ITTO in September 2014 for the creation of a laboratory in Cameroon.

  Note that the challenges of setting up a lab should not be underestimated. Once facilities and equipment have been installed, significant resources are required to maintain laboratory staff skills and equipment maintenance to the level required for effective DNA testing and analysis.

5 LESSONS LEARNED / DIFFICULTIES ENCOUNTERED

Sampling procedure

Errors in sampling led to uncertainty in geographic origin of a significant proportion of samples collected in both Cameroon and Congo.

Issues of inconsistent sampling records must be addressed in the future through additional training and the possible use of sampling applications on smartphone devices. These sampling apps are being developed by DoubleHelix and its partners to guide sampling teams through sampling instructions in real-time.

Availability of sampling consumables

It was impossible to obtain basic consumables such as silica gel and tamper proof bags locally. For the purposes of this project, silica gel was sourced and shipped from South Africa, leading to higher than necessary consumable and shipping expenses. Future project enlargement using the current consumable sources will lead to high logistics costs.

It is likely these items are used by other local industries, so with some research, local sourcing should be feasible in future.

DNA extraction performance

An average success rate of 96% for DNA extraction and amplification from log cambium was an excellent result. DNA extraction and amplification from sawn timber was lower at 69%. This rate could be improved in future with the development of additional genetic markers.

Individual versus population traceability

Individual matching was proven useful for upstream chain of custody control, and could be used by producer governments to ensure that forest concessions adhere to sustainable harvesting plans. With regards to the system of CITES controls however, comparison with the methodology applied in the *Prunus* project makes it clear that population assignment is a more simple and effective approach to trace timber back to claimed harvest areas.

Partner coordination

Significant delays were experienced during the collection of reference DNA samples from target tree populations and from saw mills due to unexpected changes in harvest and production plans by the Congo industry partner. A population assignment approach does not require the same level of coordination with industry; nevertheless better engagement and coordination with industry will be required in future.

Sample logistics

Another cause of delays to the project schedule was through the disruption of sample shipments to the University of Adelaide. A better system to authorize and approve sample shipments will be required in order for routine testing to be viable whilst local testing capacity is developed.

6 CONCLUSIONS AND RECOMMENDATIONS

- The overall project objective and key outputs were successfully achieved, although delays in sampling and subsequent DNA analysis extended the project duration from 12 to 24 months.

- The developed set of 45 SNP markers is sufficient for individual matching of log and timber samples back to tree or stump to a very high degree of confidence.

- DNA was able to be extracted and amplified from a high proportion (98%) of log cambium samples. For sawn timber samples the success rate dropped to 47%. Further marker development is recommended, although markers for population assignment can be developed from the existing pool of 358 SNP markers.

- A practical application test across two *Pericopsis* supply chains showed a breakdown in traceability based on existing chain of custody documentation, both from forest to sawmill and from log input to sawn timber output. If Governments wish to improve the monitoring of, and adherence to sustainable harvest plans in the upstream supply chain, this DNA approach can be applied.

- For the purposes of CITES controls and support of the CITES permit system, population assignment, as demonstrated in the *Prunus* project, is recommended.

- It is strongly recommended to examine the CITES permit issuing process and handling of samples for scientific purposes. A fast-track approval process is required for scientific samples. Failure to streamline the process would hinder the implementation of an effective, routine DNA verification system.

- We recommend rolling out the DNA verification system more broadly to ensure full coverage of all authorized harvest areas and areas seeking to implement a quota. Systems to monitor forest and supply chain management could be introduced prior to new quotas being assigned or as a mechanism/performance indicator to evaluate supply chain security prior to re-evaluating quotas.

- An independent party should be assigned to take samples as part of routine sampling/testing, with the support of DoubleHelix. We recommend the use of CITES authority representatives or local independent contractors.

- We recommend providing additional sampling training to local teams to ensure:
    - High level of traceability and quality sample chain of custody
    - Ease of operations and implementation
    - Pre-formatted labelling and recording tools specific to the sampling plan
    - Integration with laboratory sample identification systems for mistake proof testing
    - Fast, efficient sampling
    - Flexible sampling capable of reacting to pertinent results or changes in product order
    - Preformatted sample shipment documentation and managed logistics
    - Minimal disruption to trade

- We support the local authorities' proposal to establish local laboratories, however we advise caution due to the difficulties and costs of successfully establishing a laboratory. The cost of maintaining equipment and qualified manpower after the initial investment should not be underestimated.

- With successful implementation at small scale demonstrated through both the *Prunus* and Pericopsis projects, there is huge potential for secure the sustainable harvest and trade in other CITES-listed species around the world.

As per the Special Service Agreement (E) E14/06 and submission of this completion report, we request the disbursement of the final tranche of funds amounting to USD 26,100.

Responsible for the Report

Name: Darren Thomas

Position: CEO

Date: 20 April 2016

References

[1] Lowe, A.J., K.N. Wong, Y.-S. Tiong, S. Iyerh & F.-T. Chew. 2010b. A DNA method to verify the integrity of timber supply chains; confirming the legal sourcing of merbau timber from logging concession to sawmill. Silvae Genetica. 59: 263–268.

[2] ITTO Technical Series No. 40, Tracking Sustainability: Review of Electronic and Semi-electronic Timber Tracking Technologies, October 2012.

APPENDIX A: DNA EXTRACTION REPORT

Job number: 1087_ITO	Trial DNA Extraction Report for *Pericopsis elata*
Approved by: Dr. Eleanor Dormontt	Date: Thursday, 1 May 2014

Trial DNA Extraction Report for *Pericopsis elata*

Report written by Ms. Bianca Dunker

All laboratory and analysis work was undertaken by Ms. Bianca Dunker and Mr. Duncan Jardine, under the supervision of Dr. Eleanor Dormontt at the University of Adelaide.

Background

The University of Adelaide has been contracted to undertake a feasibility test on samples of *Pericopsis elata*, to assess whether DNA can be successfully extracted from timber. Five wood samples from different individuals were received from the Thünen Institute, Germany in April 2014. For the purposes of this analysis, the University of Adelaide accepts the species claim and has not undertaken any independent verification of the source of the samples provided.

Methodology

DNA extraction and quantification

Samples were prepared by shaving small sections of the wood with a lathe and undertaking a custom developed DNA extraction methodology (patent pending) designed for timber samples. Three replicates of each sample were included, giving a total of 15 separate DNA extraction procedures. The quantity of DNA present in each extract was measured using a Qubit® 2.0 Fluorometer (Life Technologies).

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PCR amplification

Extracted DNA from each of the five wood samples was used as a template for polymerase chain reaction (PCR) amplification of three chloroplast microsatellite loci which amplify in the majority of plants. This process was undertaken with DNA diluted to 10% (v/v) to facilitate dilution of inhibitory compounds in the solution.

Results

DNA extraction and quantification

Quantifiable DNA was successfully extracted from all three DNA extractions from each of the five wood samples (Table 1).

Table 1: DNA concentrations of the extracted samples of *Pericopsis elata*

Samples	ng/ml
1	3.47
2	2.13
3	6.63
4	3.24
5	3.47
6	2.30
7	2.24
8	9.80
9	4.42
10	3.71
11	1.80
12	3.13
13	2.11
14	2.48
15	2.71

Job number: 1087_ITO	Trial DNA Extraction Report for *Pericopsis elata*
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PCR amplification

Strong PCR product bands were obtained using the second and the third chloroplast microsatellite primer pairs (Figure 1). No amplification was present for any samples using the first primer pair. Samples from all individuals produced strong product bands. This result implies that usable DNA was successfully extracted from all samples.

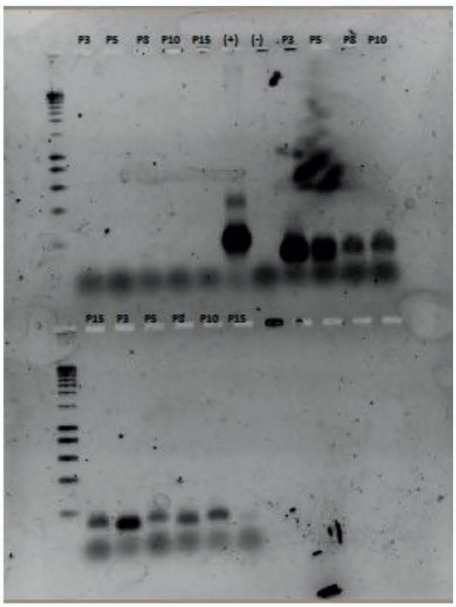

Figure 1: Agarose gel result of PCR amplification. Each column represents a single extracted sample. Sample names are abbreviated. Positive (+) and negative (-) controls were included to validate PCR procedures. A size standard ladder was used to allow PCR product size estimation from the gel.

Conclusions and Recommendations

Based on the DNA extraction trials undertaken at the University of Adelaide on samples of five individuals of *Pericopsis elata* it is our expert opinion that DNA verification of *Pericopsis elata* supply chains is feasible.

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APPENDIX B: PROJECT SAMPLING REPORT AND ANALYSIS

DoubleHelix

1087 *Pericopsis elata* sampling report

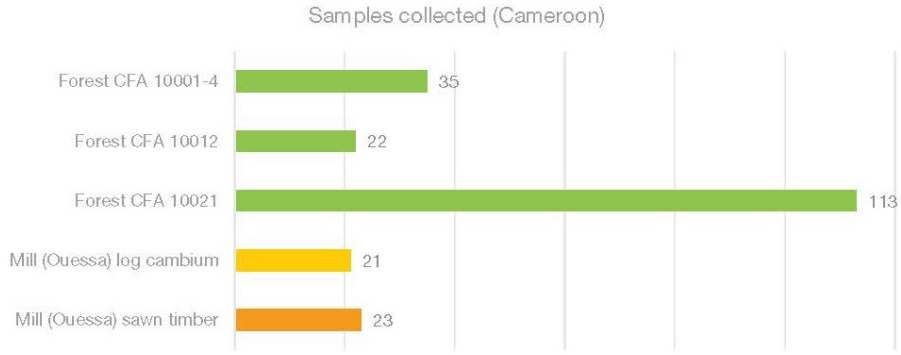
This report provides a complete description of all sample related activities related to the project. Accurate and consistent sample collection and recording proved to be a significant challenge.

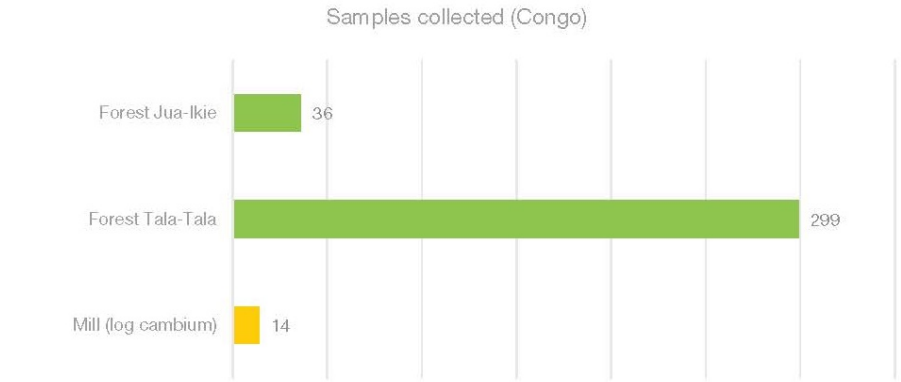
As a result, many of the provided samples were unsuitable for the development of genetic markers for individual matching and testing.

A large proportion of samples will be suitable to develop genetic markers for population assignment in the future.

Collected samples

The following breakdown demonstrates the total number of samples collected throughout the project.

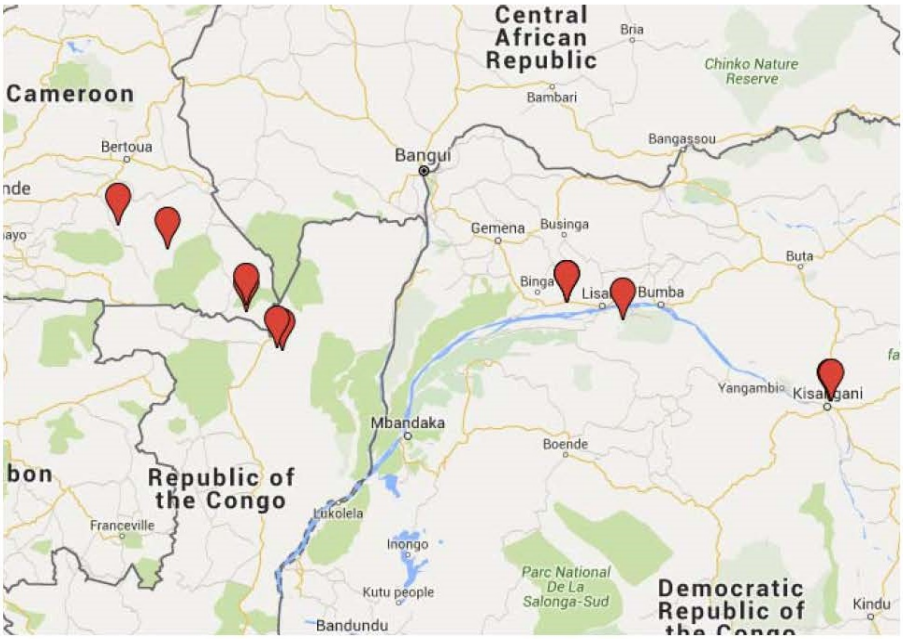




Development samples

The following shows the forest cambium samples used in marker development. Vouchered samples previously collected by the Thünen Institute of Forest Genetics were used as the development material for several reasons:

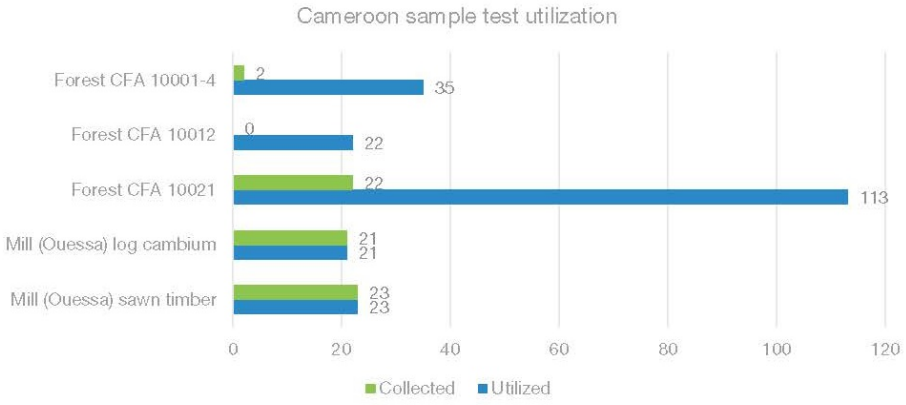
- To meet stringent requirements of meta-data recording are required for this work;
- To utilise these previous collections rather than re-collect;
- To use a spread of individuals from within and between countries with *Pericopsis elata* to capture genetic variation at different scales;
- To allow marker development to begin without the delays caused by time taken to collect samples and obtain permits.

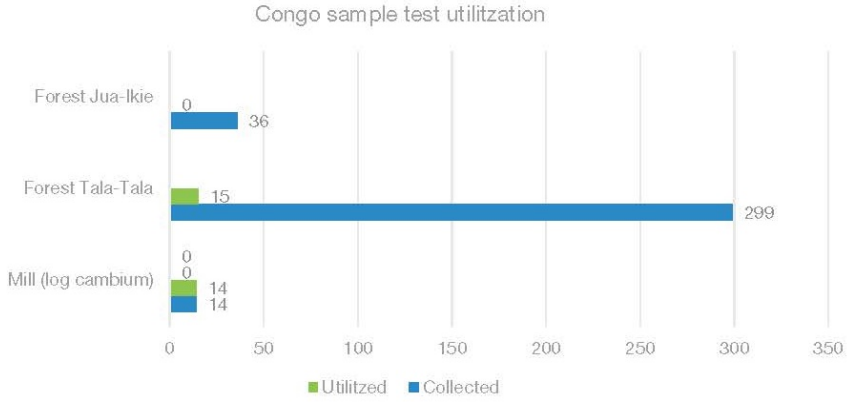


Location	Total
Cameroon	4
Congo	2
DRC	7
Total	**13**

Test samples

The following shows the samples utilized for testing the developed markers vs the total collected samples. The intended test was to match 50 paired samples of cambium and sawn wood totaling 100 samples. 97 samples were used comprised of a combination of forest cambium, forest wood, sawmill cambium and sawmill wood.





Meta-data that would establish required location claims was missing for 130 of the collected Congo forest cambium samples and were thus unusable in the project.

Data which would connect paired samples of wood and cambium from the same tree at different points in the supply chain was also missing or inaccurately recorded in many cases. For 107 of the Cameroon forest cambium samples coordinates were not properly recorded.

Lastly a large delay in sample collection and delivery at the sawmill level rendered 27 of the sawn wood samples from Congo unusable as work had already proceeded to meet project the completion deadline of end March 2016.

The tested samples still provide adequate verification of the effectiveness of the developed markers to pair sample of the same individual tree.

Map of all samples collected during this project (excluding Thünen Institut)

Key

? Sample populations which are missing data or were not recorded reliably

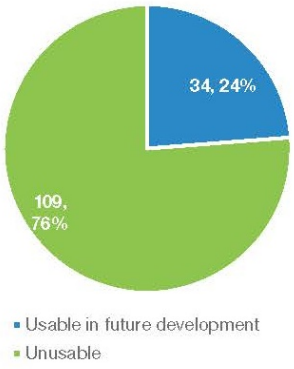
Sample population 1 Cameroon covering CFA 20021, 20012, 2001-4

Sample population covering UFA Tala Tala

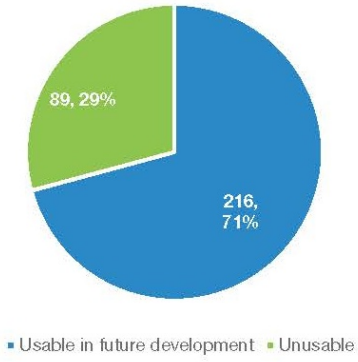
Sample population covering UFA Jua Ikie

Some samples which were properly collected and recorded were also not utilized in the project due to repetition of data or cost restrictions. These samples can still be utilized in the future for further population marker development. The below graphs demonstrate which of those samples unused in testing and development at this stage have all the requisite meta-data and are in viable condition to be utilized in future work.

Cameroon



Congo



APPENDIX C: FINAL LAB REPORT FROM THE UNIVERSITY OF ADELAIDE

Job number: 1087_ITO	Final Report for *Pericopsis elata*
Approved by: Professor Andrew Lowe	Date: Monday, 18 April 2016

Final Report for *Pericopsis elata*

Report written by Dr. Eleanor Dormontt

All laboratory and analysis work was undertaken by Dr. Rainbo Belton, Dr. Bianca Dunker, Dr. Korjent van Dijk and Ms Dona Kireta, under the supervision of Dr. Eleanor Dormontt and Professor Andrew Lowe at the University of Adelaide.

Background

Based on the successful feasibility study which confirmed that DNA of sufficient quantity and quality can be extracted from the wood of *Pericopsis elata*, The University of Adelaide has been contracted to develop a suite of genetic markers for *Pericopsis elata*, for Chain of Custody (CoC) verification.

Methodology

Samples used for genetic analyses

Detailed information on the samples that were collected as part of this project and specifically those which were used for genetic analysis, can be found in the accompanying sampling report. For the purposes of this technical report, basic sample information is summarised in table 1.

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Table 1. Summary of samples used for genetic analyses in *Pericopsis elata.* For further information consult accompanying sampling report.

Purpose	Number of samples	Type of samples	Geographic origin of samples
SNP Development	13	Leaf	Cameroon (4), Congo (2), DRC (7)
Forest sampling	54 (from 39 trees)	Cambium (39), wood (15)	Cameroon (24), Congo (15)
Mill sampling	58	Cambium (35), wood (23)	Congo (14), Cameroon (43)

DNA extraction and quantification

Samples for the project were received either as leaf, cambium, or wood (table 1). DNA extractions for leaf and cambium samples were undertaken using the Nucleospin Plant II Kit (Machery-Nagel, Düren, Germany) with the PL2/PL3 buffer system. DNA was extracted from wood using a patented methodology licenced to Double Helix Tracking Technologies (University of Adelaide). All samples were quantified on the QuantiFluor® dsDNA System (Promega).

DNA marker development and screening

Thirteen samples collected in 2008/2009 by the Thünen Institute (table 1) were used to develop single nucleotide polymorphism (SNP) markers in *Pericopsis elata*. SNP discovery was made using double digest RAD-Seq (ddRAD), a modified approach of (Vos et al. 1995) using the Mi-Seq sequencing platform (Illumina). Once identified through ddRAD, the SNPs were optimised for use on the MassARRAY iPLEX platform (Agena Bioscience) (Gabriel et al. 2009; Oeth et al. 2005) and all forest and mill samples (table 1) were screened in this way. Thirty-seven samples were replicated with DNA extraction and MassArray genotyping undertaken twice ('technical replicates'), in order to assess the repeatability of the genetic analyses. Negative control samples and extractions blanks were included to ensure the genotypes are attributable to the DNA samples not contamination.

Data analyses

The raw genotyping data for all samples were analysed. All loci which failed to amplify in all samples or did not present polymorphic genotypes within the sample set were excluded from further analyses. The technical replicate samples and negative controls were used to assign an appropriate loci-amplification threshold, allelic mismatch threshold, and to assess the overall error rate of the genetic test.

Validation of approach with known samples

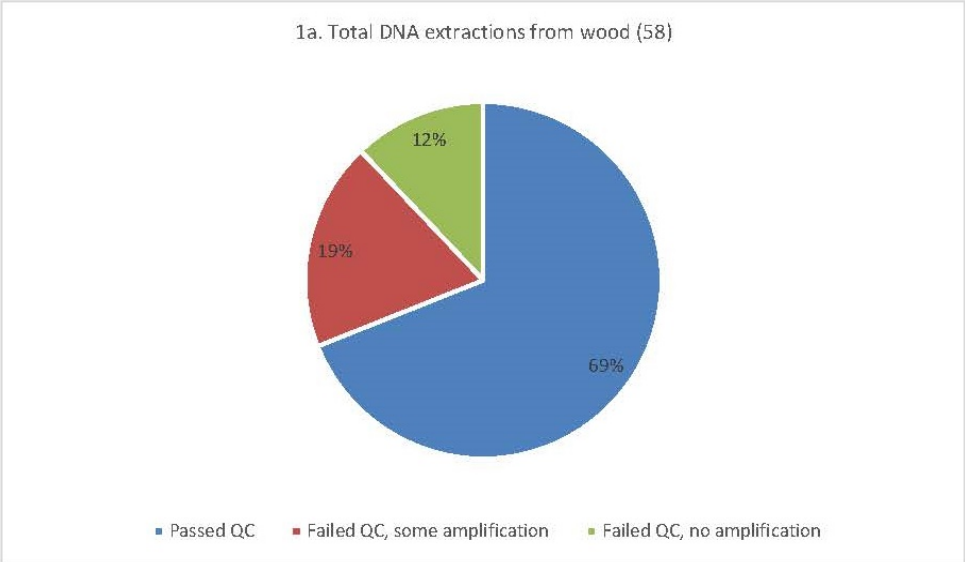
The allelic mismatch threshold was set to 2, meaning that two paired samples may show a maximum of two single allele differences in their genotypes and still be considered matches. These mismatches must be where one sample shows a homozygote and the other shows a heterozygote. If any samples show alternate homozygotes at a locus, they are not assigned as a match. This mismatch threshold is a conservative measure allowing for rare potential genotyping errors which can occur in degraded samples such as timber.

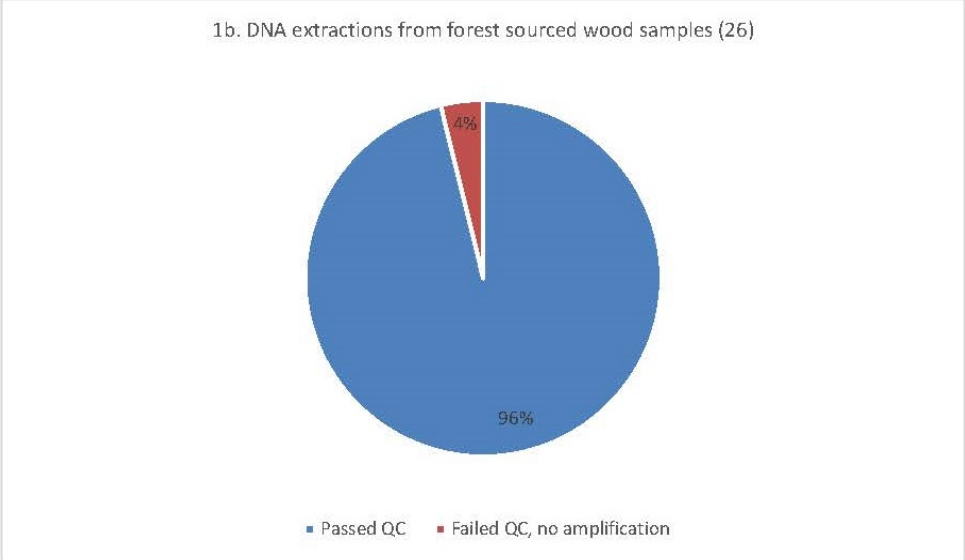
Table 2. Summary of samples that produced genotypes which passed quality control thresholds in *Pericopsis elata*.

Purpose	Number of samples	Type of samples	Geographic origin of samples
Forest sampling	47 (from 35 trees)	Cambium (35), wood (12)	Cameroon (23), Congo (12)
Mill sampling	46	Cambium (32), wood (14)	Congo (14), Cameroon (32)

DNA extractions

In total, 58 wood extractions were completed, comprised of 26 forest samples and 32 mill samples. Overall, 69% of wood extractions passed quality control (Figure 1a). The greater proportion of these were the forest samples, of which only one failed. Of the mill samples, only 47% passed QC, 31% showed some loci amplification but not enough to pass QC, and 22% showed no amplification at all. Of the 88 total cambium DNA extractions, 6 failed completely and 7 had some amplification but failed QC, giving an overall success rate of 85%.





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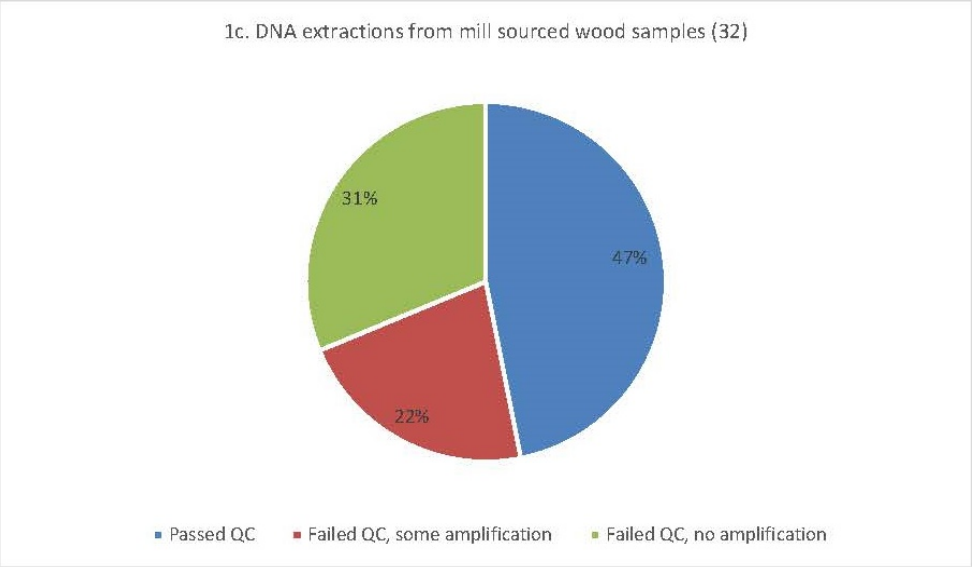

Figure 1. Pie charts showing the outcomes of DNA extraction from wood samples in the project. 1a shows all results combines, 1b refers to forest sourced wood samples only and 1c refers to mill sourced wood samples only.

Validation of approach with known Congo wood samples

Twenty-two wood samples (from 11 trees) matched 100% to their cambium pair, demonstrating that on genuinely matched sample pairs, the genetic test returns 100% congruence between samples derived from the same tree.

Comparison of mill samples with putatively matching forest samples

In the Congo, cambium samples from 14 logs taken at the sawmill prior to processing were compared to the reference samples for the claimed trees taken when they were standing in the forest. Eleven mill samples matched their forest counterparts (79%), the remaining three (21%) did not match their claimed counterpart nor any other of the genotyped samples in the project (Figure 2).

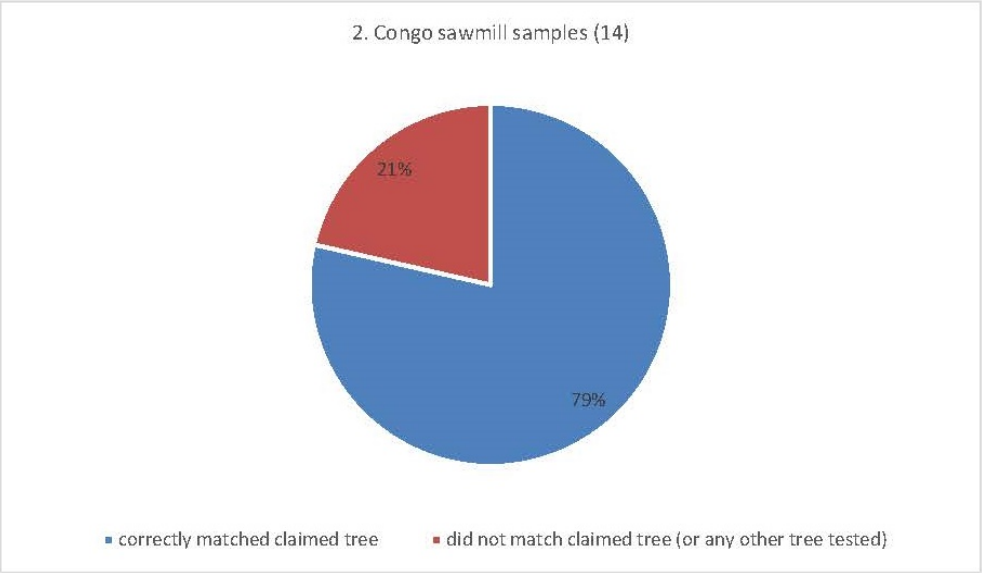

Figure 2. Pie chart showing the sawmill to forest cambium sample matches in the Congo.

In Cameroon, cambium samples from 18 logs taken at the sawmill prior to processing were compared to the reference samples for the claimed trees taken when they were standing in the forest. Thirteen mill samples matched their forest counterparts (72%), 4 samples did not match their claimed forest sample but instead matched other forest samples which had been successfully genotypes, the remaining one (6%) did not match their claimed counterpart nor any other of the genotyped samples in the project (Figure 3).

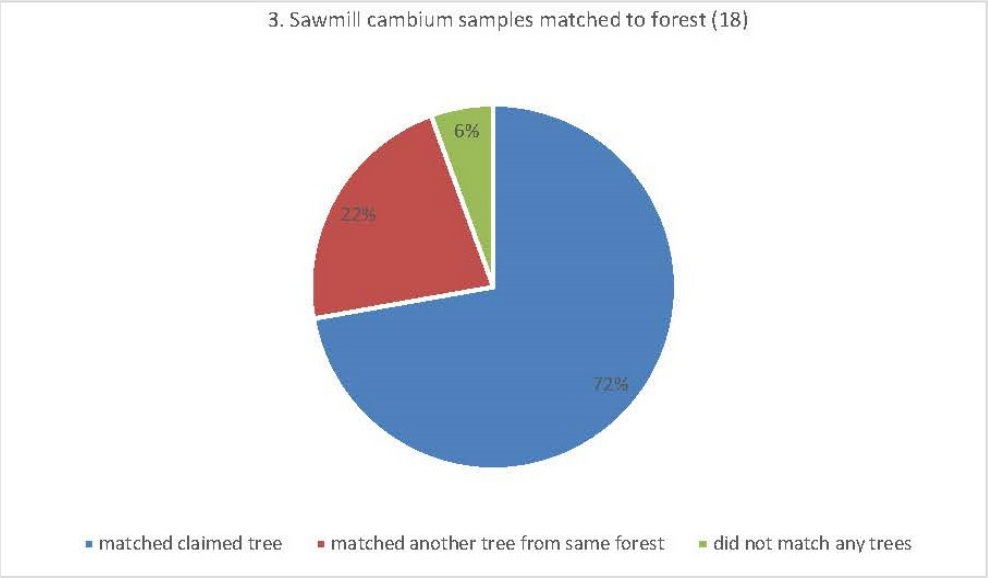

Figure 3. Pie chart showing the sawmill to forest cambium sample matches in Cameroon.

The combined data for both the Congo and Cameroon cambium to sawmill matching are shown in figure 4. A total of 14 samples from the Congo and 15 samples from the Cameroon were tested and matched back to their supposed tree of origin in the forest. Overall the results were slightly better in the Congo (79% vs 72%). An additional four trees from the Cameroon matched trees that were different from the claim, but were still from the same forest. When these samples are taken as positive matches, the Cameroon results improve and exceed those of the Congo (94%).

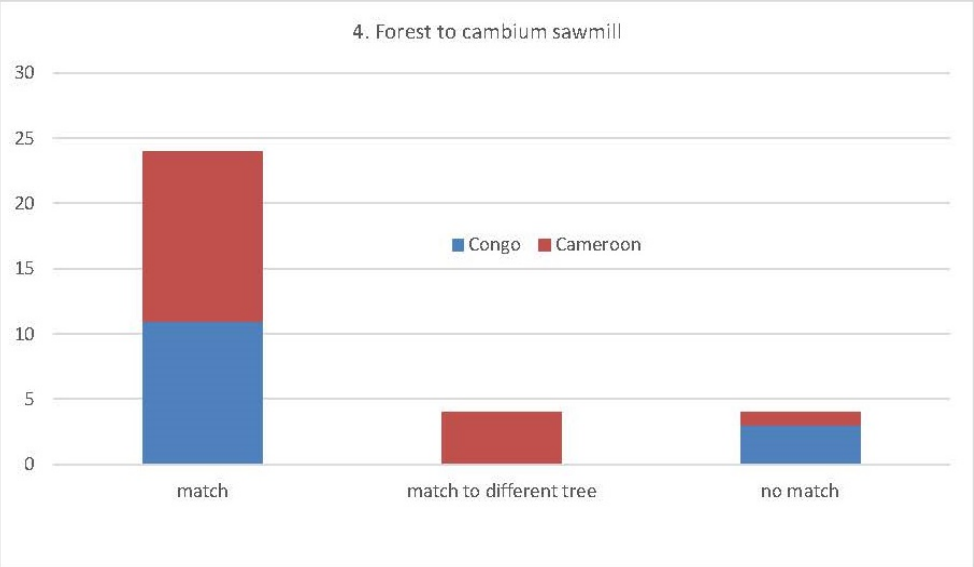

Figure 4. Combined forest to sawmill cambium sample matching data for the Congo and Cameroon

Comparison of sawn timber mill samples with putatively matching cambium mill samples

In the Cameroon, 14 sawn timber samples were taken from the mill and a claim was made that they matched cambium samples also taken at the mill prior to processing. The specific identity of each tree was not claimed, just that as a whole, the genotypes should match those collected previously. Of these 14 samples, three samples (21%) matched an individual as claimed, the remaining 11 (79%) did not match any samples successfully genotyped as part of the project (figure 5).

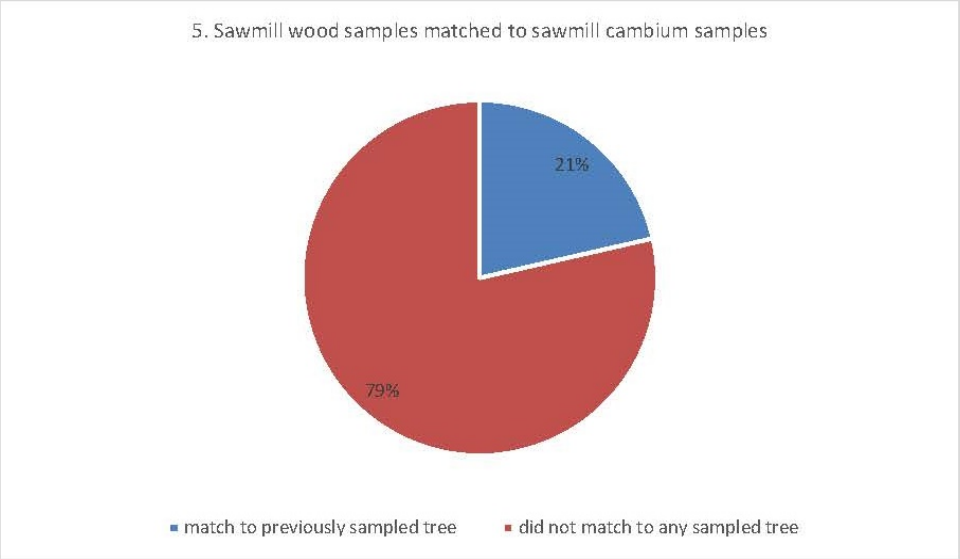

Figure 5. Pie chart showing the sawmill wood to sawmill cambium sample matches in Cameroon.

Random match probabilities

In unrelated individuals, where all loci are used for comparison, the random match probability is 1 in 392,727,556,596 (approximately 390 billion). For related individuals (siblings), the random match probability is 1 in 798,583 (approximately 800,000). As it is possible that trees in the same concession are related, this second random match probability provides a more conservative estimate. The exact random match value for a specific comparison will depend on the number of loci that were successfully genotyped in each sample and thus could be directly compared (table 3). The lowest random match probability for any sample comparison in this study was 1 in 7714 (approximately 8000).

Table 3. Random match probabilities for related and unrelated individuals of *Pericopsis elata* using up to 45 loci developed in this study.

Number of loci compared	Random match probability (unrelated) one in…	Random match probability (siblings) one in…
1	2	2
2	6	3
3	16	4
4	17	4

5	22	5
6	24	5
7	56	8
8	125	12
9	253	17
10	563	26
11	1269	39
12	1343	40
13	3280	63
14	7490	95
15	16026	140
16	42146	233
17	104582	372
18	223120	544
19	371764	699
20	839839	1056
21	1459421	1387
22	2017504	1627
23	5001837	2601
24	8070332	3292
25	13446820	4233
26	14231499	4355
27	37616701	7284
28	39877719	7499
29	42204753	7714
30	61290667	9270
31	142350404	14226
32	257465911	19065
33	323069220	21329
34	424482767	24405
35	908265229	35734
36	1132632277	39855
37	2794715952	63515
38	7383036609	106197
39	14375071278	147824
40	33155183612	225923
41	76470313018	345284
42	199010555356	570856
43	222706114966	603668
44	235701961289	620972
45	392727556596	798583

Conclusions

- A set of 45 SNP markers for chain of custody verification in *Pericopsis elata* have been developed.
- Of the processed timber samples tested, 47% of extractions produced DNA sufficient for successful genotyping. Cambium extractions were successful 85% of the time.
- Validation of the method on known matched cambium and wood samples showed 100% matches between pairs of samples.
- Technical replicates showed no genotyping errors, giving an error rate of less than 0.1% per locus or less than 0.05% per allele.
- In the Congo, 79% of cambium sawmill samples matched their claimed forest sample, in Cameroon this value was 72%.
- In the Cameroon, an additional 22% of cambium sawmill samples matched forest trees that were different to those claimed.
- In Cameroon, 21% of processed timber samples matched their claim, the remainder did not match any individuals genotyped in the study.
- The random match probability for all 45 loci in unrelated individuals is approximately 1 in 390 billion, in related individuals it is approximately 1 in 800,000.

Recommendations

At present, the current set of markers are suitable for chain of custody verification in *Pericopsis elata* and can be utilised to accurately determine the individual identity of *P. elata* samples, including processed timber. This final set of 45 loci were chosen from a pool of 358 putative markers for *P. elata*. Given the range of individuals used for initial marker development and the number of loci that were not polymorphic in the small dataset used in this study, there is a very high probability that within this pool of remaining 313 SNP markers, there are loci which can facilitate population level identification in *P. elata*. We recommend that the project be extended to appropriately assess the suitability of the available markers for population level assignment, as this would facilitate identification of origin without the requirement for multiple samples to be collected.

References

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Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic acids research **23**:4407-4414.

APPENDIX D: LIST OF PROJECT STAKEHOLDER MEETINGS

DoubleHelix

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28/1/2016

Stakeholder meetings list

Name	Job title	Organization	Date of	Purpose of meeting
Dr Betti Jean Lagarde	ITTO regional coordinator	ITTO	23 April 2014	Exchanges on the projects
NKOUM Yves	senior staff (assistant to pericopsis project coordinator	ANAFOR	6 and 12 May 2014	Exchanges on the projects and project opening workshop
OWUSU Njombe	Deputy general director (assistant to prunus project coordinator	ANAFOR	6 and 12 May 2014	Exchanges on the projects and project opening workshop
Dr Betti Jean Lagarde	ITTO regional coordinator	ITTO	14 May 2014	exchanges for clarifications on certain points of the projects
NKOUM Yves		ANAFOR		
OWUSU Njombe	Deputy general director (assistant to prunus project coordinator	ANAFOR		
Persons from stakeholders		ANAFOR	23 May 2014	Pericopsis national technical committee
		ANAFOR	18 June 2014	review and prospective for coming activities
OWUSU Njombe	Deputy general director (assistant to prunus project coordinator	ANAFOR		
NKOUM Yves		ANAFOR	14 July 2014	Exchanges on material, approaches...
OWUSU Njombe	Deputy general director (assistant to prunus project coordinator	ANAFOR		
BEKOLO BEKOLO	General Director	ANAFOR		
Persons from stakeholders		ANAFOR, MINFOF, AFRIMED, timber industry, universities, research institute	22 July 2014	Prunus project National technical committee
OWUSU Njombe, NKOUM Yves		ANAFOR	26 Sept 2014	coordinating with assistants coordinator from ANAFOR on sampling at enterprises level, first phase...
OWUSU Njombe		ANAFOR	3 Oct 2014 and 14 Jan 2015	presentation of the project and workplan to the local staffs of MINFOF and national park
EBENG EBAI	MINFOF sud-west regional delegate	MINFOF		
Samplers		interns		
Korup National park representatives		Korup		
MBONGO Martin	Responsible for CITES permits issuing	MINFOF	17 Oct 2014	exchanging with people from MINFOF on the projects
NGOY TAKI Pascal	Prunus project responsible in DRC	MECNT	30 Oct 2014	coordinating on sampling ahead of new field phase
Afrimed mill team		AFRIMED	27 Dec 2014	being at the mill to explain and coordonate the sampling to come
Persons from stakeholders		ANAFOR, MINFOF, AFRIMED, timber industry, universities, research institute	27 April 2015	Prunus project National technical committee
Persons from stakeholders		ANAFOR, MINFOF, AFRIMED, timber industry, universities, research institute	30 Dec 2015	Prunus project National technical committee

APPENDIX E: FINANCIAL REPORT

N°	OUTPUT / ACTIVITY	TOTAL (US$)	EXPEND-ITURES	Notes
10.	**Output 1.1 Development of genetic markers for *P. elata***			
	11. Activity 1.1.1 Trial DNA extraction	1 500	1 500	
	12. Activity 1.1.2 Population sampling (Cameroon and Congo)	15 000	12 271	Lower laboratory fees due to weaker AUD compared to USD
	13. Activity 1.1.3 DNA marker development	40 000	23 271	
	14. Component Total	**56 500**	**37 042**	
20.	**Output 1.2 Capacity building and training of local teams**	**-**	**-**	
	21. Activity 1.2.1 Workshops for stakeholder consultation and field training	ITTO	5 851	Opening workshops/training only
	22. Component Total	**ITTO**	**5 851**	
30.	**Output 1.3 Implementation of DNA verification**			
	31. Activity 1.3.1 Verification system development	8 000	7 500	
	32. Activity 1.3.2 Field implementation	17 500	16 000	AUD / USD exchange rate change during the course of the project
	33. Activity 1.3.3 Ongoing DNA testing	30 000	24 514	
	34. Activity 1.3.4 Statistical analysis	**-**	**-**	
	35. Component Total	**55 500**	**48 014**	
40.	**Project coordination and reporting**			
	41 Lead consultant	16 200	24 300	Additional coordination time due to change in sampling schedule and associated overheads
	42 Project manager	19 500	12 405	
	43. Administrative support	8 400	20 210	
	43. Component Total	**44 100**	**56 915**	
100.	**GRAND TOTAL**	**156 100**	**147 822**	