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 *Prunus africana* in Prunus Allocation Units in Cameroon and Democratic Republic of Congo (DRC).

PERIOD OF REPORT	01 May 2014 to 30 January 2016
PROJECT NUMBER	PP-A/39-162A
EXECUTING/IMPLEMENTING AGENCY	Ministry of Environment, Nature Conservation and Tourism (MECNT), Democratic Republic of Congo. Ministry of Forestry and Wildlife (MINFOF), Cameroon
COLLABORATING AGENCIES	Double Helix Tracking Technologies, Singapore Syndicates of Industries in charge of Harvesting, Processing and Exportation of Special Products (SIHPESP) in Cameroon and Democratic Republic of Congo.
DURATION	22 months
START DATE	March 2014
PROJECT BUDGET	US$ 360 675
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 ITTO-CITES project coordination team have been evaluating tracking systems that can provide reliable identification of products produced in concessions/forests where improved management standards have been implemented and the non-detriment findings required by CITES to allow legal exports have been undertaken. This pilot project was selected to show that DNA verification can detect and deter substitution or addition of *Prunus* bark from non-authorised sources, and increase supply chain transparency. Through this approach it is hoped that an increase in export quotas can be considered based on scientific data that provides hard evidence of secure supply chains.

The original project objective was to implement an effective DNA-based traceability system that would allow *Prunus africana* bark to be traced back to *specific trees* from controlled PAUs. Mid-way through the project in March 2015, a change to the scope of work was requested based on a better understanding of ground operations and operational limitations in DRC. The approved change meant that additional work could be undertaken to develop traceability back to distinct populations (or geographic areas) of *Prunus*, as well as to individual trees. This work was conducted at no extra cost to ITTO.

All project outputs and activities were successfully carried out, including additional sampling and laboratory activities required under the expanded scope of work. The project duration was extended from 18 to 22 months to allow for the additional work, and also due to initial delays in sampling, delays in issuance of CITES permits for scientific samples and impounding of samples by customs authorities.

Tangible outputs from the project include a suite of 16 genetic markers that can be used to assign samples back to both individual trees and populations (distinct geographic regions such as PAUs or harvest zones) with a high degree of confidence. Genetic reference data for 8 *Prunus* populations has been developed which can be utilized for traceability purposes. Raw genetic sequence data generated by this project can also be used to support other research efforts such as landscape reconstruction or climate change adaptation for the species. A comprehensive DNA verification system has also been designed that allows for routine verification of CITES permit applications, export permits and import permits.

Key lessons learnt were in relation to improving field sampling practices to avoid errors in collection and sample recording. It is also now clear that population assignment is a more useful technique to apply where chain-of-custody procedures are either non-existent or lacking. However assignment of bark to individual trees is viable in areas such as Mount Cameroon, where good chain of custody procedures already exist. Many difficulties were encountered with the existing CITES permit application process and shipment of samples, even as part of this scientific exercise.

This project has proven that randomly selected samples can be matched back to a PAU or harvest zone of origin, or an individual tree if necessary. A DNA verification system has been proposed that can verify claims of origin on CITES export and import permits to be applied at multiple ‘control points’ along the *Prunus* supply chain. Before it can be implemented, 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.

In future, further genetic reference data can be easily established for other PAUs and harvest zones that would enable blanket coverage of all authorized *Prunus* exploitation and secure the trade in sustainably harvested *Prunus* bark. The same approach can be applied to safeguard the sustainability of other CITES listed tree species around the world.

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)." Within the programme, the project supports 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 project is consistent with both DRC's and Cameroon's Forest policies.

The project is the latest in a series supported by the ITTO focusing on 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, designed to verify Chain-of-Custody documentation of merbau (*Intsia* spp.), processed for export to Australia, New Zealand and Europe and supported by ITTO through Activity No.: PP-A/43-194 - Promote Trade in Tropical Timber and Tropical Timber Products from Sustainably Managed and Legally Harvested Sources). Project results have been published in the peer-reviewed scientific journal Silvae Genetica[1] and a recent ITTO-CITES publication[2].

Other ITTO funded DNA projects have included PD 620/11 Development and Implementation of a Species Identification and Timber Tracking System in Africa with DNA Fingerprints and Stable Isotopes, which involved genetic mapping of multiple tree species across seven countries in Africa.

This project is therefore the latest to demonstrate the practical application of DNA technology and its real-world implementation. It offers a real study of impact on forest and sawmill operations and demonstrates that the likely running costs of such a system is a small percentage of the final *Prunus* product value.

1.2 Origin and problem

The ITTO-CITES project coordination team had been looking for tracking systems that can provide reliable identification of products produced in concessions/forests where improved management standards have been implemented and the non-detriment findings required by CITES to allow legal exports have been undertaken.

Suspected *Prunus* exports from non-project areas trying to pass off as from better managed areas or trying to piggy-back on quotas established and approved for the better managed areas added further impetus to the implementation of this project.

It is hoped that through the successful implementation and roll out of this project, better monitoring of controlled sustainable *Prunus* supply chains will lead to an increase in supply chain transparency and confidence, allowing for an increase in export quotas to be considered based on scientific data that provides evidence of secure supply chains.

2 PROJECT OBJECTIVES AND IMPLEMENTATION STRATEGY

2.1 Objectives and adjustments

The original project objective was to implement an effective DNA-based traceability system that would allow *Prunus africana* bark to be traced back to *specific trees* from controlled PAUs.

The proposed DNA traceability system would enable random verification of traceability documentation and CITES export/imports permits to detect bark substitution from uncontrolled areas and associated document fraud to a high level of confidence, allowing for timely corrective actions to be implemented.

Adjustments

Mid-way through the project in March 2015, a change to the scope of work was requested based on a better understanding of ground operations and operational limitations in DRC.

Due to difficulties in bark segregation in DRC and ongoing security concerns, DoubleHelix recommended adjusting the proposed system to allow for traceability back to distinct *Prunus* populations (PAUs or harvest zones), rather than to individual trees. Whilst we would no longer be aiming to identify the specific tree that a piece of bark came from, we would be able to identify and verify that bark came from the claimed PAUs or harvest zones. This capability would still enable independent, scientific verification of CITES export permit applications.

2.2 Implementation strategy and adjustments

The implementation strategy consisted of:

Preparation and development of DNA reference data

- Preliminary work to confirm the ability to extract DNA from *Prunus* bark. If unsuccessful, this would have been a severely limiting factor to successful project completion.

- In parallel with the above, we worked to train sampling teams in both Cameroon and DRC in proper procedures for sample collection, recording, storage and shipment. This training was combined with the opening workshops, with practical field trips conducted in the days following the workshops in both countries.

- After training, an initial round of sampling of *Prunus* from standing trees in different populations in Cameroon and DRC was conducted by the sampling teams.

- These samples were sent to the Thünen Institute for analysis and development of genetic markers (a process call ‘genotyping’) that enables discrimination between different trees of the same species.

Field implementation

- Further discussion and research was conducted into *Prunus* harvesting, transportation and processing activities, in order to build knowledge that would inform the development of the DNA-based verification system. The basis of the system is simple: apply DNA testing to verify and validate claims of origin of harvest in CITES export and import permits.

- A field implementation stage was planned to determine how samples could be taken during actual harvest and processing operations. At this point we determined that in DRC, bark is mixed too early in the process to maintain segregation between trees. This prompted a change in approach to population assignment rather than individual tree assignment.

DNA testing and analysis

- Final DNA testing and statistical analysis was then conducted at the Thünen Institute in Germany to determine the effectiveness of the approach to assign samples taken from warehouses and processing facilities back to PAUs and harvest zones.

2.3 Risks and assumptions

The key risk identified during the proposal phase was security in East DRC. At the time the Government of DRC had recently negotiated a peace deal with rebels in the north and South Kivu, allowing for field work to take place. Sampling in this area was in fact disrupted, with a batch of bark samples confiscated by local militia, and repeat sampling undertaken.

A further risk was possible problems of DNA extraction from *Prunus* bark which would require additional efforts to develop genetic markers. This risk was mitigated through a trial DNA extraction activity at the beginning of the project, and successful analysis against existing genetic markers of *Prunus persica.*

Assumptions were made that there would be no problems associated with the transportation of bark samples from Cameroon and DRC to the laboratory in Germany, or with existing harvest and processing procedures that would allow for document matching of bark back to individual trees.

3 PROJECT PERFORMANCE

Based on the performance levels detailed below and presentation of project deliverables, DoubleHelix considers the project objective of building the capability to trace *Prunus africana* bark back to specific PAUs or harvest zones to a high level of confidence to be achieved.

With the adjustment to the scope of work, the original objective has been exceeded, considering it is now possible to assign samples back to both individual trees and populations. Either approach can now be applied, depending on which approach is practical in each country or supply chain.

3.1 Achievement of project outputs and activities

There was some variation between the original planned outputs and activities which resulted in the successful completion of the project. A comparison between the project outputs and activities as originally devised and the final outcomes follows:

Output 1.1: Development of genetic markers for *Prunus africana* suitable for DNA fingerprinting of bark (differentiation of bark between individual trees of the same species).

Activity 1.1.1 Trial DNA extraction from bark samples

Project document description	Initial tests will be performed on *Prunus africana* bark samples to confirm that DNA of sufficient quality and quantity can be extracted from bark on a routine basis.
Changes to activity	None
Realized activity	Complete. A preliminary test to verify that DNA can be extracted from *P. africana* bark 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 is presented in **Appendix A**.

Activity 1.1.2 Population sampling

Project document description	Two hundred (200) samples will be collected from a number of different populations within the species range. Twenty (20) samples each from 5 different populations across Cameroon and another 5 different populations in DRC will be collected.
Changes to activity	This activity was extended with additional collections in DRC to build capability for assignment to population as well as individual matching.
Realized activity	Complete. A total of 600 samples were collected across four populations in Cameroon and five populations in DRC. There was unclear geographic separation between collections in Cameroon and DRC, and these populations were therefore combined. It is likely this happened due to incorrect recording of sample coordinates. In addition, a population from Kenya was included in the genetic analysis to provide further comparison between populations. For more detail on the sample quantities and locations, refer to **Appendix B**. DNA sample records were provided in the mid-term project report delivered December 2014.

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	Whilst the number of markers to be developed was not specified in the original project description, it was decided to increase the number of markers developed from 10 to 15 in order to build capability for population assignment.
Realized activity	Complete. A total of 36 different *Prunus* SSR markers (31 nuclear and 5 chloroplast SSR markers) were tested for amplification and segregation in the available *Prunus africana* populations. 16 markers (15 nuclear and 1 chloroplast SSR markers) were used in the final analysis. Based on these markers, it was possible to identify 9 distinct *Prunus africana* populations from the samples submitted. All populations from Cameroon form one genetic cluster and all populations from East-Africa (DRC + Kenya) form another cluster. Of interest is that the four populations from Cameroon are split into two distinct genetic groups. The two populations (Cam Pop1 and Cam Pop4) in the South-West of Cameroon are very different from all the other populations. For more detail on the marker analysis, refer to **Appendix B**.

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	We recommend 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 trade company representatives in Cameroon and DRC, government representatives, customs representatives and international buyers of *Prunus* bark. The opening workshop will include a field trip to conduct training of staff in bark sampling techniques and can also be combined with the population sampling (Activity 1.1.2).
Changes to activity	To save costs, we recommended combining workshops with the similar *Pericopsis elata* project.
Realized activity	Two opening workshops completed. Closing workshop completed in Yaounde, Cameroon on 2 Feb 2016, with a further closing workshop in DRC planned for March 2016. 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.

Output 1.3: Implementation of DNA verification in two controlled PAUs in Cameroon and DRC from pre-harvest to the factory and then to the exit points.

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 standing trees and bark. 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, transport and processing 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 bark substitution amounting to 1% of total volume, 99% of the time").
Changes to activity	No change
Realized activity	Complete. A number of phone calls and meetings have been held with members of ITTO, ANAFOR, MINFOF, MECNT and industry and academic stakeholders. See **Appendix C** for a list of stakeholder meetings held during the project. Final recommendations for a DNA-based verification system are presented in **Appendix D** and are to be discussed in detail at the closing meetings.

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 *Prunus africana* trees either prior to harvest or during harvest. ii) A second set of samples are taken from bark after harvest at an appropriate control point. Control points under consideration could be one or more of i) PAU exit gate ii) community drying or storage areas, iii) on delivery to processing facilities, iv) prior to loading onto trucks for transport to the factories, v) at the port prior to export or vi) on arrival at destination processing facilities in Europe. iii) Using CoC documentation, the samples taken pre- and post-harvest are physically matched together. iv) A statistically representative number of paired 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	This has changed significantly due to the change in work scope toward population analysis. Paired sampling and testing was conducted on a portion of samples in Cameroon and DRC. Another set of samples from Cameroon, DRC and Kenya were tested and assigned to population.
Realized activity	Samples for individual assignment were taken from one site in Cameroon (AFRIMED), consisting of 55 pairs of cambium and bark, and a site in DRC (KAHINDO) consisting of 150 pairs of cambium and bark. Of these, 30 pairs from Cameroon and 60 pairs from DRC were tested. All remaining samples were collected from PAUs and harvest zones in DRC to be tested and assigned to population. For further detail, see **Appendix B**.

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 and bark 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. A genetic mis-match indicates that the paired samples come from different trees. This is evidence of substitution and further action can be taken.
Changes to activity	This activity was expanded to include both testing for individual assignment and population assignment.
Realized activity	Both individual and population assignment tests were completed. Individual assignment revealed difficulties in successfully matching materials from bark back to tree. Population assignment was comparatively easy and more successful (see Activity 1.3.4). For further detail see **Appendix B**.

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	This activity was expanded to include analysis of both individual assignment and population assignment.
Realized activity	*Individual assignment* The practical test with material processed at a mill in Cameroon showed a high level of mis-match between results and chain of custody records of bark from individual trees. Thus we recommend focusing further applications on verification of the population of harvest. *Population assignment* Genetic differences between populations are highly significant and the success of assignment back to the correct population was achieved with a mean average of 95%. For further detail see **Appendix B**.

3.2 Comparison of activity progress against schedule

Table 2 below shows the project implementation in comparison with the detailed workplan provided to ITTO in May 2014 and revised in March 2015. The approved workplan schedule is marked in grey, with the actual implementation indicated by the hatched red cells.

Table 2: Activity schedule in comparison with workplan

OUTPUTS / ACTIVITIES	RESPONSIBLE PARTY	Month																						
		May 2014	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2015	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2016	Feb
Output 1.1																							
1.1.1 Trial DNA extraction	DoubleHelix																						
1.1.2 Population sampling	MECNT, ANAFOR, DoubleHelix																						
1.1.3 DNA marker development	DoubleHelix																						
Output 1.2																							
1.2.1 Workshops and training	MECNT, ANAFOR, DoubleHelix																						
Output 1.3																							
1.3.1 Verification system development	DoubleHelix																						
1.3.2 Field implementation	MECNT, 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 223,716

This figure consists:

  - o USD 177,930 of direct expenses
  - o USD 45,786 of project coordination and reporting costs

This figure does not include estimated expenses for attendance at the proposed project closing meeting in DRC in March 2016.

The increased expenditure reflects the expansion in the scope of work and extension of project duration from 18 to 22 months. The additional cost has been absorbed by DoubleHelix at no charge to ITTO. For further detail on expenditure against budget (original and revised), see **Appendix E**.

- Total funds received from ITTO to date: USD 160,000

- Remaining funds to be released: USD 36,100

4 PROJECT OUTCOME

The objective of building capability to trace *Prunus africana* bark back to specific PAUs or harvest zones to a high level of confidence has been realised. The approach implemented in this pilot project can be expanded to other authorized PAUs and harvest zones as part of efforts to control and support the sustainable harvest and trade in *Prunus* bark.

Attempts to sample and match *Prunus* bark from warehousing and processing facilities back to specific trees were challenging, even in cases where it was possible to segregate bark from different trees during harvest and transport.

It is fortunate therefore, that the scope of work was adjusted to allow an implementation of population assignment to take place. Table 3 below clearly illustrates the effectiveness of this approach, where on average 95% of samples were assigned to the correct population.

Table 3: Results of the self-assignment of individuals. The yellow cells mark the percentage of correctly assigned individuals

	% assigned to								
	Cam pop1	Cam pop2	Cam pop3	Cam pop4	DRC pop2	DRC pop3	DRC pop4	DRC pop5	Kenia
Cam pop1	95			5					
Cam pop2		100							
Cam pop3			100						
Cam pop4	3			97					
DRC pop2					94	2	2		2
DRC pop3		2				90	3	3	2
DRC pop4						7	87		6
DRC pop5						5		95	
Kenia									100

There was some significant challenges to be overcome during the course of the project, which is described in detail in **Section 5**.

4.1 Tangible outputs

A suite of genetic markers

36 different *Prunus* SSR markers (31 nuclear and 5 chloroplast SSR markers) have been developed, with 16 of them (15 nuclear and 1 chloroplast) used for the final project analysis. These markers can be used for both individual assignment (matching of bark back to individual trees, as well as population assignment (matching of bark back to a geographic area). As well as their utility for bark traceability, these markers have many other potential research applications as described below.

Genetic reference data for 8 distinct Prunus *populations across Cameroon and DRC*

For the first time, genetic profiles have been created for specific regions of *Prunus africana*. The data generated has an extremely long lifetime and will be valid for traceability purposes for generations to come. The raw sequence data generated during this project can be shared with other research institutes and scientists for other analysis such as landscape reconstruction and climate change adaptation.

Recommendations for a system to support verification of CITES applications, export and import permits.

As described in **Appendix D**, DoubleHelix has proposed a DNA verification system that can be used to verify the accuracy of CITES export permit applications and the export/import permits themselves at key control points in the supply chain.

As forest management practices develop in PAUs and harvest zones under the ITTO-CITES programme, the DNA verification system can be adapted to monitor improvements in the evolving document chain of custody requirements.

4.2 Participation of target beneficiaries

- Training of sampling teams in Cameroon and DRC on sampling techniques, sample packing, storage and logistics. Participants in the training included those from local academic institutions, universities, Government departments and *Prunus* exploitation industry.

- Participation of industry in sampling process at PAUs and warehouse/distribution centres.

- Ongoing communication with *Prunus* exporters in DRC and importers in the EU.

- Discussion with CITES authorities on potential implementation of systems in Cameroon.

Refer to **Appendix C** for details on meetings with stakeholders describing participation in the project.

4.3 Next steps

At the request of the European Commission SRG, a paper has been prepared by DoubleHelix on recommended activities to follow on from this project.

In order for a traceability system to be of full benefit, it must be applied to the total coverage area of *Prunus* exploitation. Our recommendations include:

- Peer review and publication of the scientific genetic marker development work.

- Additional sampling and marker development to enlarge the genetic reference database to include an additional 15 PAUs and harvest zones across the region.

- 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 areas, as described in **Appendix D**.

- Create a fast-track CITES permit issuing system for scientific samples to ensure speed of delivery of test samples from Cameroon and DRC 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 DRC.

Whilst the geographic coordinates of Cameroon Pop1 and Pop4 did not show a clear geographic separation of the samples of both populations, genetic analysis showed significant genetic differences among these sets of individuals. This was because Pop4 was a subset of Pop1, taken from a very limited area. In fact, this sampling demonstrated the ability to discriminate between two populations within the same PAU, only 15km apart.

In DRC, the populations 1a and 1b, the supplied GPS coordinates did not show a clear geographic separation of the samples of both populations. Moreover both populations did not show a clear genetic separation in the first population genetic analyses. For that reason both populations were combined to form Pop1.

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

Availability of sampling consumables

Surprisingly, it was impossible to obtain basic consumables such as silica gel and tamper proof bags locally. With better support, it should be possible to source these items locally, which are likely to be used by other local industries. For the purposes of this project, silica gel was sourced and shipped from South Africa, leading to higher than necessary consumable and shipping expenses.

DNA extraction performance

An average success rate of 50% for DNA extraction from dried bark is satisfactory, but could be improved in future with the development of additional markers. This has been included in the proposal for a second project phase.

Individual versus population traceability

During the opening workshops, we were able to research and understand the supply chain mechanism of *Prunus* in Cameroon and DRC. The originally envisaged system of bark-to-tree matching would work if procedural changes are made to harvesting, bundling and storage processes in collaboration with local community workers and industry. This approach is likely to work with PAUs in the Mount Cameroon area.

In DRC however, bark harvesting and collection is carried out by a large and diverse number of groups and coordination between these groups and conformance to a specific procedure is not possible. In this case, population assignment is more appropriate.

Timing

Minor delays were experienced during the collection of reference DNA samples from target tree populations due to wet weather (wood samples should not be collected during wet weather since moisture in samples can lead to mould and sample contamination).

Security

Security of sampling teams in DRC was a significant concern. On one occasion, the sampling team was forced to give up *Prunus* samples and camera equipment to bandits. In addition to the threat to safety of the sampling team, an additional sampling mission was required to make up for the lost samples.

This has been addressed to some degree by focusing on population assignment, which eliminates the requirement to return to harvest zones on a regular basis for tree sampling. Instead, reference samples for each harvest zones need only be collected once, limiting travel and exposure in unsafe areas.

Sample logistics

The main cause of delays to the project schedule was through the disruption of sample shipments from both Cameroon and DRC to Germany. For unspecified reasons, there were delays in CITES export permit approval and import permit approval.

DRC sample populations 3-5 were held by German customs for several weeks in Oct-Nov 2015, severely delaying processing and analysis of samples. As a substitute for these missing populations, an additional population from Kenya, was included into the population genetic analysis, just in case the DRC samples were confiscated indefinitely.

These sorts of delays will make routine testing of samples impossible within acceptable time limits in the future, and must be addressed before routine DNA verification can be put into practice.

6 CONCLUSIONS AND RECOMMENDATIONS

- All project outputs were successfully achieved or exceeded. Whilst this required additional resources and an extension of the project duration from 18 to 22 months, the additional costs were absorbed by DoubleHelix and at no cost to ITTO.

- The developed set of 16 SSR gene markers is sufficient for the assignment back to the individual and population of harvest. For a broader application we recommend to add 5 to 10 gene markers to compensate fragmented data sets due to DNA-degradation in the bark. On average for 50% of the bark samples we have sufficient DNA quality and thus a sufficient number of successfully amplified gene markers.

- Genetic differences in the gene marker set were highly significant among all pairs of populations and the success of assignment back to the population was very high, with a mean average of 95%. Assignment was successful even in harvest areas only 15km apart (Pop1 and Pop4 in Cameroon; Pop3 and Pop5 in DRC). This clearly demonstrates the success of DNA to verify PAU or harvest zone origin.

- The practical application test with *Prunus* bark chips showed a high level of errors in the chain of custody of bark from individual trees. We recommend focusing future efforts on verification of the population of harvest. This approach better fits the objectives of the ITTO-CITES project without disruption to established harvesting processes. The potential for individual assignment back to tree can be used in future if steps are taken to monitor harvest of correct trees as part of continually improving forest management standards.

- 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. Since the volume of samples for testing is low compared to commercial shipments, we see no practical reason why a separate CITES authorization and approval process should not be developed for scientific samples. DoubleHelix is willing to discuss this together with ITTO, the CITES Secretariat and the Executive Agencies. Failure to implement such a process would severely hinder the implementation of an effective 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, enhanced sampling training 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 recommend 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 proof of concept demonstrated through this *Prunus* project, there is huge potential for application to other CITES listed species around the world as part of the ITTO-CITES programme to protect and create a truly sustainable trade in such species.

As per the Special Service Agreement (E) E14/14, we request the disbursement of the final set of funds of USD 36,100.

Responsible for the Report

Name: Darren Thomas

Position: CEO

Date: 10 February 2016

References

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

APPENDIX A: DNA EXTRACTION REPORT

First analysis of 5 *Prunus africana* samples using SSR markers from *Prunus persica*

Background

The Thünen-Institute of Forest Genetics tested the DNA extraction from cambium and bark of *Prunus africana* and the application of known SSR markers of *Prunus persica* for genotyping in *Prunus africana.*

Methodology

Sample preparation and DNA isolation

The Thünen-Institute of Forest Genetics received 5 samples of *Prunus africana* bark (PrAf1 to 5, Fig. 1).

Fig. 1: *Prunus africana* samples (after sample preparation)

All surfaces of the samples were incubated under UV light, each side for 2 hours. To minimize contaminations with foreign DNA (e.g. fungi) parts of the outer layer of the samples, surrounding the area of sample preparation, were removed, using a sharp scalpel. All together 20 different samples were prepared for DNA isolation (4 different samples were taken from every piece of bark). For cambium preparation (samples labeled with "k") the inner layer of the bark samples was scraped off and $^1/_3$ to $^1/_2$ of a 2ml reaction tube was filled with the resulting shavings. For bark preparation (samples labeled with "a", "b" or "c") the outer and radial layer of the bark samples was scraped off and $^1/_3$ to $^1/_2$ volume of a 2ml reaction tube was filled with the resulting shavings. Two steal balls (diameter 5 mm) were added to each prepared sample, and all samples were frozen in liquid nitrogen and grinded in a Retsch mill (Retsch GmbH, Haan, Germany) 3 times for 100s using a frequency of 17,5 Hz.

The DNA of the samples was isolated according to the custom developed wood DNA isolation protocol using 900µl of extraction buffer, 45µl DTT and 18µl proteinase K. The incubation in extraction buffer was performed overnight at 55°C.

The prepared DNA was solved in 20 µl of water and stored at -20°C. For the determination of DNA concentration and quality all samples were measured using the Nanodrop spectrophotometer (Thermo/ Fisher Scientific, Schwerte, Germany). The results of the spectrophotometric analysis are given in table 1.

Sample ID	ng/ul	A260	A280	260/280	260/230
PrAf1a	6,3	0,126	0,188	0,67	0,17
PrAf1b	8,44	0,169	0,225	0,75	0,17
PrAf1c	3,82	0,076	0,097	0,79	0,15
PrAf1K	-170,08	-3,402	-0,598	5,69	1,21
PrAf1K	-11,18	-0,224	0,044	-5,09	80,64
PrAf2a	19,06	0,381	0,574	0,66	0,13
PrAf2b	20,61	0,412	0,594	0,69	0,12
PrAf2c	3,67	0,073	0,091	0,81	0,25
PrAf2K	61,31	1,226	1,054	1,16	0,33
PrAf2K	2929,62	58,592	13,899	4,22	1,46
PrAf3a	225,27	4,505	6,36	0,71	0,41
PrAf3b	42,72	0,854	1,216	0,7	0,18
PrAf3b	44,07	0,881	1,279	0,69	0,18
PrAf3c	63,23	1,265	2,269	0,56	0,14
PrAf3K	115,12	2,302	0,913	2,52	0,61
PrAf4a	3,88	0,078	0,118	0,66	0,19
PrAf4b	4,27	0,085	0,078	1,1	0,22
PrAf4c	22,18	0,444	0,971	0,46	0,1
PrAf4K	6,84	0,137	0,24	0,57	0,15
PrAf5a	56,33	1,127	0,968	1,16	0,36
PrAf5b	39,6	0,792	0,645	1,23	0,51
PrAf5c	89,53	1,791	1,687	1,06	0,37
PrAf5K	112,99	2,26	1,099	2,06	1
H2O	0,77	0,015	0,001	16,14	1,1

Tab. 1.: Nanodrop analysis of the *Prunus africana* samples. Listed samples with identical sample IDs are due to repeated measurements of the same sample.

After Nanodrop analysis the samples were purified using the UltraClean 15 DNA Purification Kit (MoBio, Carlsbad, USA, sold by Dianova GmbH, Hamburg, Germany).

SSR marker analysis

Isolated DNA of all 5 original samples was used for SSR PCR testing. 5 SSR markers (Pr. UDP98_411; Pr. UDP98_412; Pr. UDP96-001; Pr. UDP96_018; Pr. UDP98-022) developed for *Prunus* persica (Testolin *et al.* 2000) were used for PCR reactions, under standard PCR conditions. Each forward primer carried a fluorescent tag. Thermal cycling was performed with denaturation and annealing steps of 30s and 45 s of elongation time. Cycle number was 33. PCR reactions were run on an ABI 3730 DNA capillary sequencer.

Results

Quantifiable DNA was successfully extracted from all 5 *Prunus africana* samples. Fig. 2 shows an example of a PCR-test of 2 *Prunus persica* SSR-markers with the genomic DNA from *Prunus africana*. Both primers amplified well and different alleles could be identified. This indicates that DNA extraction from *Prunus africana* cambium and bark samples is possible and the application of markers developed for other *Prunus* species is suitable for *Prunus africana*.

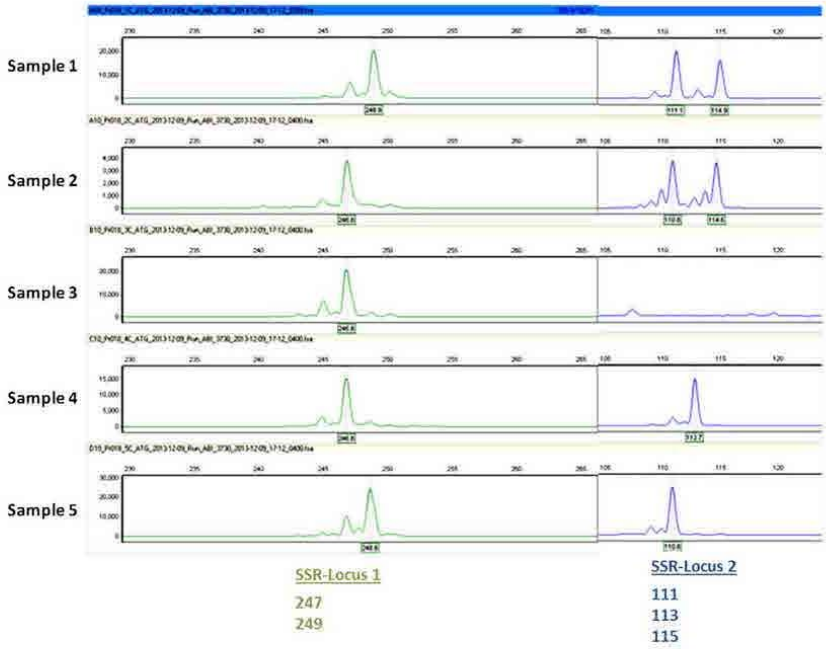

Fig. 2: Example of a PCR-test of 2 *Prunus persica* SSR-markers with genomic DNA from *Prunus africana*

Literature

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APPENDIX B: FINAL LAB REPORT FROM THÜNEN INSTITUTE

Genetic assignment of *Prunus africana* samples from different African countries

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(Last changed: 26/01/2016)

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Objective

The objective of the work was to develop and test a set of gene markers that is suitable to assign bark of *Prunus africana* back to the individual and population of harvest.

Sample material/ Populations

Different populations of *Prunus africana* were sampled in Cameroon and the Democratic Republic of Congo. An additional population from Kenia from a former project at the Centre for Ecology and Hydrology (CEH) in Edinburgh, UK, was included into the analysis. Fig. 1 gives an overview of the geographic locations of the sampled populations.

Fig. 1: Locations of the populations

Cambium samples of different individuals (with known geographic coordinates) within a population were taken with a punch, dried in silica gel and shipped to the Thünen Institute of Forest Genetics for DNA extraction.

Cameroon

4 different populations were sampled in Cameroon (Fig. 2). The geographic coordinates of Pop1 and Pop4 did not show a clear geographic separation of the samples of both populations. But the genetic analysis came out with significant genetic differences among both sets of individuals. The unclear geographic separation of the two populations may be due to mistakes in the documentation of the geographic coordinates.

Fig. 2: Locations of the populations from Cameroon

DRC

5 different populations were sampled in the Democratic Republic of Congo (Fig. 3). The populations 1a and 1 b did not show a clear geographic separation of the samples of both populations. Moreover both populations did not show a clear genetic separation in the first population genetic analyses. For that reason both populations were combined to form Pop1.

The populations 3-5 were stuck in German customs for several weeks. Pop3 and Pop5 seem to be located in the same region as Pop1, but in contrast to Pop 1a and b, both populations are geographically clearly separated.

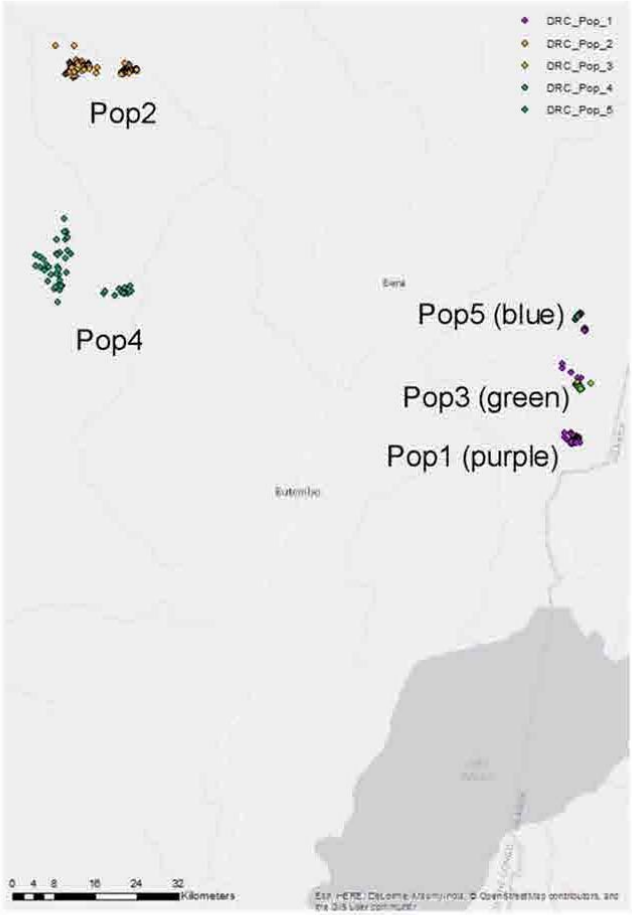

Fig. 3: Locations of the populations from DRC

Kenia

As a substitute for the missing populations 3-5 from DRC that were blocked several weeks at the customs in Germany, an additional population from Kenia, was included into the population genetic analysis.

Tab. 1 gives an overview of the populations, which were included in the genetic analysis.

Country	Population	No of individuals	No of individuals tested	No of individuals in analysis
DRC	1	100	100	99
DRC	2	150 pairs (bark/cambium)	60 pairs	56
DRC	3	43		43
DRC	4	48		48
DRC	5	44		44
Cameroon	1	40	40	39
Cameroon	4	55 pairs + 110 single individuals	30 pairs (mill sample assignment)	30
Cameroon	2	35	35	31
Cameroon	3	30	30	30
Kenia	Kibiri	274	274	54

Tab. 1: Overview of the populations used in the population genetic analysis so far

Methods Laboratory

DNA extraction

DNA extraction was carried out using a modified version of the protocol of Dumolin et al. (1995).

Marker analysis

The marker analysis was based on the use of microsatellite (SSR) markers. Primer sequences were gained from various publications (Ciprianai et al 1999, Sosinski et al. 2000, Vaughan and Russel 2004, Ohta et al. 2005, Chester et al. 2007, Cavers et al. 2009). Moreover various *Prunus SSR* primers already available at the Thünen Institute of Forest Genetics were tested for usability in *Prunus africana*. SSR marker analysis was carried out using PCR amplification of the marker fragments using either labelled forward-primers or tailed forward-primers in combination with labelled tail-primers. PCR reactions were then separated on an ABI 3730 DNA Analyzer capillary sequencer. Fragment analysis was carried out using Gene Marker V2.4.0 software. A total of 36 different *Prunus* SSR markers (31 nuclear and 5 chloroplast SSR markers) were tested for amplification and segregation in the available *Prunus africana* populations. 16 markers (15 nuclear and 1 chloroplast SSR markers) were used in the final analysis.

Population genetic analysis

With the program GDA_NT (Degen unpublished) we analyzed the genetic diversity and differentiation among populations. For the further analysis we left the population 1 from DRC out because the geographic positions of the trees overlapped with the positions from DRC populations 3 and 5. A total of 171 different alleles and haplotypes were observed at all loci. The mean effective number of alleles varied between A_e = 2.4 and A_e = 3.6. These values are for microsatellites low compared to the values of other tree species.

The commonly used measures for genetic differentiation among all populations were very high: delta $_{Gregorius}$=0.34, F $_{ST\ Hedrick}$=0.45 (Gregorius 1996, Hedrick 2005). Based on the allele/ haplotype frequencies at all 16 loci we computed genetic distances (Gregorius 1996) among all pairs of populations and visualized the distance matrix in a cluster analysis. As tested with a permutation test (data not shown) the genetic distances among all pairs of populations were statistically significant (p> 0.99). The dendrogram (figure 4) shows the result of the cluster analysis.

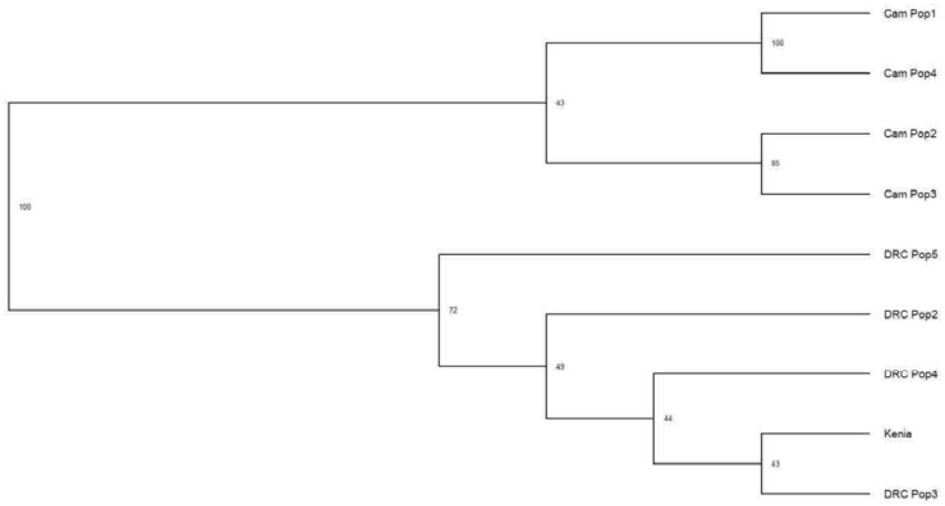

Fig. 4: Dendrogram of genetic differences among the gene pools of the 9 *Prunus africana* populations. The numbers are the results of the bootstrapping over all alleles / haplotypes and indicate the statistical level of significant differences.

In the dendrogram we see two genetically different main groups. All populations from Cameroon form one cluster and all populations from East-Africa (DRC + Kenia) form another cluster. Interesting is that the four populations from Cameroon are spited in two groups. The two populations (Cam Pop1 and Cam Pop4) in the South-West of Cameroon are very different from all the other populations.

Self-assignment

Individual assignment tests were performed using the Bayesian multilocus-approach (Rannala and Mountain 1997). All individuals from the reference data were self-classified to the sampled populations using the leave-one-out approach (self-assignment, Efron 1983). The proportion of correctly assigned individuals estimates the precision of the approach (tab. 2). All individuals with data for at least 10 gene loci were included. The results were very good. On average 95% of the individuals were assigned to the correct population.

	% assigned to								
	Cam pop1	Cam pop2	Cam pop3	Cam pop4	DRC pop2	DRC pop3	DRC pop4	DRC pop5	Kenia
Cam pop1	95			5					
Cam pop2		100							
Cam pop3			100						
Cam pop4	3			97					
DRC pop2					94	2	2		2
DRC pop3		2				90	3	3	2
DRC pop4						7	87		6
DRC pop5						5		95	
Kenia									100

Tab. 2: Results of the self-assignment of individuals. The yellow cells mark the percentage of correctly assigned individuals

Mill sample assignment

Part of the project was to test the possibility of assigning bark samples of *Prunus africana* sampled during different steps of product processing back to the tree of origin.

In a first step 30 bark samples, harvested before product processing, which were supposed to belong to 30 known individuals, which were part of Population 3 from Cameroon, were sent to the Thünen Institute of Forest Genetics. Marker analysis of the 16 SSR markers was carried out for all 30 bark samples. Marker results within the pairs, which consisted out of 1 cambium sample (part of Pop3) and one of the bark samples were compared with each other.

Altogether 16 out of 30 pairs were identified to consist out of identical individuals. Unfortunately 6 pairs were identified to consist out of non-identical individuals, pointing towards sampling errors. For 8 pairs the results remained unclear, either due to low DNA quality and low marker amplification rates of at least one of the two individuals (7 pairs) or due to unclear marker results (1 pair).

In a second step 12 bags of bark pieces, sampled after milling of the 30 trees used in step 1, were sent to the Thünen Institute of Forest Genetics. 2 pieces of bark were selected from every bag (24 samples). The DNA was isolated and marker analysis was carried out as described above.

Comparison of the 24 bark samples with the 30 bark/cambium pairs resulted in

- 8 cases in which an assignment of the post milling bark sample to an individual of the 30 bark/cambium pairs was possible (some were assigned to individuals belonging to incorrect or unclear pairs)
- 12 cases in which no assignment was possible due to low DNA quality and low marker amplification rates
- 4 cases in
- which the post milling sample matched none of the analyzable individuals included in the 30 pairs

Conclusions

- The developed set of 16 SSR gene marker is sufficient for the assignment back to the individual and population of harvest. For a broader application we recommend to add 5 to 10 gene marker to compensate fragmented data sets due to DNA-degradation in the bark.
- On average for 50% of the bark samples we have sufficient DNA quality and thus a sufficient number of successfully amplified gene markers.
- Genetic differences at the gene marker set were highly significant among all pairs of populations and the success of assignment back to the population was with a mean of 95% very good.
- The practical application test with material that get processed at a mill in Cameroon showed a high level of errors in the chain of custody of bark from individual trees. Thus we recommend focusing in further applications on verification of the population of harvest.

Literature

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APPENDIX C: LIST OF PROJECT STAKEHOLDER MEETINGS

DoubleHelix

Germain_List of meeting_prunus_001
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 D: DNA VERIFICATION SYSTEM DESIGN

Prunus DNA verification system

Prunus africana harvest system

The commercial harvest of *Prunus* bark for export in Cameroon and DRC is controlled by their respective federal departments overseeing forestry and the regulations agreed upon through their adherence to CITES. Each country has a different strategy to managing the *Prunus* trade. This strategy largely impacts the structure and functional operation of the trade and supply chains. The goals of these management systems are the same:

1. Control areas and volumes of *Prunus* bark harvest to ensure a sustainable industry.
2. Control *Prunus* exports to only include bark harvested from legal sustainably managed sources.

Cameroon

Cameroon's *Prunus* exports accounted for an average of 38% of global trade between 1995 and 2004, rising to 72.6% (658.6 tons) in 2012. Cameroon exports both un-processed dried bark and processed bark meaning bark which has been crushed into small pieces and powder. The total export quota for 2015 was 1082 tons.

Table 1 outlines the basic supply chain of *Prunus* harvest and export in Cameroon along with the required documentation and oversight of MINFOF for both CITES and domestic regulatory controls.

Table 1

Supply Chain	Actions	CITES/MINFOF control
In the forest	• PAU established by MINFOF, local company, foreign buyer	
	• Management plan developed for PAU	• MINFOF validates management plan
	• Annual harvest inventory	• Trees are tagged and documented (DBH, Coordinates) *
	• Harvest	• Controller reports harvested tree number, volumes on MINFOF doc
Transport	• Harvested bark collected at central point in forest for shipment to mill/warehouse	• Volume, origin, destination are recorded on MINFOF waybill
In the mill/warehouse	• Bark is offloaded, dried, bagged at this point if un-processed • Processed and bagged if powder	
	• Bags are loaded into a container for export • Containers sealed • Containers loaded onto truck for transport	• CITES export permit requested. Volume, origin, and recipient all must be provided. • CITES export permit and certificate of origin issued
At the port	• If check is required, containers are sealed for export	• Information requests filed at the port accompanied by official CITES document package.

* Not mandatory to tag every tree with GPS coordinates except in Mt Cameroon area.

DRC

DRC accounts for roughly 16% of global *Prunus* trade. Unlike Cameroon all of DRC's exported bark is in the form of un-processed dried bark.

Table 2 outlines the basic supply chain of *Prunus* harvest and export in DRC along with the required documentation and oversight of MECNT for both CITES and their domestic regulatory controls.

Table 2

Supply Chain	Actions	ICCN/CITES/MECNT control
In the forest	• Zone identified for *Prunus* exploitation • Harvest from *Prunus* in official zone • *Prunus* is cut, dried and bagged in forest	• Inventoried and mapped by exporter • Exports controls are *Prunus* in that zone
Transport	• Bagged *Prunus* shipped to central facility	
Exporter facility	• Harvested bark collected at central facility controlled by exporter • *Prunus* de-bagged and checked for quality • Buyer contract for a specific volume • Bark is re-bagged and loaded onto a container	• CITES export permit applied for declaring zone origin and volume • Once granted foreign buyer applies for import permit covering same bark volume
Transport	• Container is sent to Mombasa, Kenya for export	
At the port	• Container is unloaded and reloaded to a new container and shipped	• Export volumes restricted by CITES quota (72 tons).

DNA verification systems

As proven in the ITTO pilot we now have the capacity to verify the origin of *Prunus* bark back to its origin of harvest. The following system description and implementation plans demonstrate the structure of a DNA verification system designed to support and strengthen CITES supply chain controls while minimizing disruption to trade. These systems will provide unprecedented supply chain transparency and material chain of custody for a CITES restricted species.

Complete supply chain verification

Sampling will be administered across the total annual volume exported by each trader under the current quota system. Samples will be collected for testing at two place as seen in images 1 and 2. Screening samples at these two points is integral in creating complete transparency.

The claim of origin submitted in the CITES permitting process will be verified by results from the first testing point. Results from the second testing point at import will ensure the bark covered by the CITES permit is the same bark delivered to the buyer. Not only does this provide transparency from forest to final import if red flags are found it will also show where in the supply chain the fraud was introduced.

Image 1 Cameroon sampling points for DNA verification

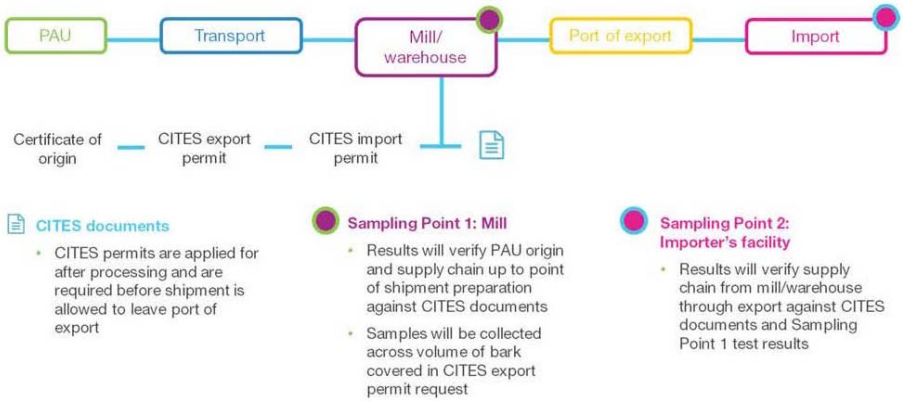
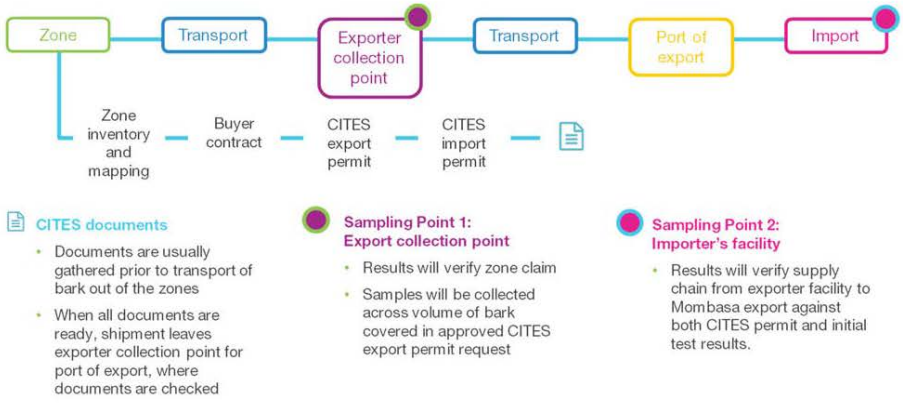


Image 2 DRC sampling points for DNA verification



Sampling

Sampling should be conducted by an independent party with the support of DoubleHelix. This could be a MINFOF or CITES authority representative or a third party representative. DoubleHelix has well developed established procedures for conducting independent, verifiable and reliable sampling. We can provide training and support 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 your 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

Screening

Testing a statistically relevant, predetermined number of samples pulled from product shipments over a given period of time is the best way to realistically verify the claims associated with that product. DoubleHelix has developed an algorithm to help determine the appropriate number of samples to apply to a population of product, in this case the annual volume of *Prunus* exported by a single exporter.

Our algorithm is built to produce confidence intervals as a measure of how strongly the sample testing results are correlated to the overall population. This means the percentage of falsely claimed results found in the sample population can be proportionally related to the total population. See table 3 for example. Because the algorithm's statistical models adhere to a normal distribution curve there is typically an upper limit of 75. This means that typically testing more than 75 samples does not improve the correlation between sample results and the overall product population.

As such we recommend 75 samples be tested across the total annual export volume for each exporter. How those 75 samples are distributed between the two sampling points is flexible depending on multiple factors including:

- Length of supply chain after the first sampling point
- Risk of fraud in the supply chain
- Previous testing results
- The number of PAUs or Zones from which the bark in a particular shipment was harvested

This flexibility ensures the best use of sample resources and accounts for risks particular to each exporters supply chain.

Table 3

75 samples across a population will determine intervals of the percentage of false claims in the entire product order, based on the number of tests with results that find a false origin claim (observed frequency). This is done with a 95% confidence level.

Observed frequency	Confidence interval (% of potential false claims in population)
25%	15%-35%
15%	5%-25%
10%	0.2%-18%
3%	Up to 9%

Example of *Prunus* screening system

Using historical data we can provide an example of how DNA screening can be implemented in the Cameroon *Prunus* export. As at 2013, Afrimed was by far the largest exporter of *Prunus* in Cameroon, followed by Pharmafric. In the absence of data on how shipments are received at port, we will use the 2013 annual volumes to define screening populations for demonstration purposes.

Afrimed exports 482 tons of dried bark per year. For highest statistical certainty at lowest cost, we recommend conducting 76 tests. At EUR 300 each testing across this tonnage would cost EUR 22,800 (USD 24,889.28). The value of this export volume is USD 2.9 million (USD 6000 x 482 ton). Hence this screening program will cost only 0.89% of the total export value.

Sampling implementation

For the purposes of this example Afrimed harvests all its *Prunus* from the Mount Cameroon area. This means the complexity of the supply chain is relatively low. Sampling will be focused at the mill level of processed bagged bark ready for shipment and awaiting CITES export permit approval and certificate of harvest. A smaller portion of the 76 will be taken at the import point as there is less risk of fraudulent material between the mill and export from Douala. Table 4 demonstrates the sampling plan.

Product description

Annual Volume	Description	Claims for testing
482 tons or 19 containers	• Processed 50 Kilo bags of *Prunus africana* chips	Origin verification • Mt Cameroon PAUs

Sampling plan

76 tests distributed across the two points of sampling based on supply chain assessment. Barks will be randomly selected from different containers and bags first when processing is complete at the mill in Cameroon and second at the point of import once received by the buyer.

Product	Sampling plan at mill	Sampling plan at import
Afrimed *Prunus*	• 3 samples per container ready for shipment • 57 total	• 1 sample per container received • 19 total

Implementation

- **At the mill** - When product is ready for loading and shipment and a CITES permit has been applied for 3 samples are taken from three random bags for every container, (or representative container volume) included in the shipment for export covered by the requested CITES permit.
- **At import** - When product is received 1 sample is taken from 1 random bag of each container.

All samples will be sent to The Thünen institute in Germany. DoubleHelix can support logistics of shipment. Testing will take one month from samples received to results. Interpretation of results is critical in making accurate connection to issues in the supply chain. DoubleHelix can support this process as well through results reporting.

APPENDIX E – FINANCIAL REPORT

N°	OUTPUT / ACTIVITY	TOTAL (US$)	REVISED	EXPEND-ITURES	Notes
10.	**Output 1.1 Development of genetic markers for *Prunus africana***				
	11. Activity 1.1.1 Trial DNA extraction	1 500	1 500	1 500	
	12. Activity 1.1.2 Population sampling (Cameroon and DRC)	15 000	15 000	20 000	Additional costs of field sampling in DRC after change in project scope
	13. Activity 1.1.3 DNA marker development	80,000	90 000	90 000	
	14. Component Total	**96 500**	**106 500**	**111 500**	
20.	**Output 1.2 Capacity building and training of local teams**	**-**	**-**	**-**	
	21. Activity 1.2.1 Workshops for stakeholder consultation and field training	ITTO	ITTO	9 000	DoubleHelix staff travel for workshops and training was not budgeted for. This item for opening workshops/training only
	22. Component Total	**ITTO**	**ITTO**	**9 000**	
30.	**Output 1.3 Implementation of DNA verification**				
	31. Activity 1.3.1 Verification system development	8 000	5 000	5 000	
	32. Activity 1.3.2 Field implementation	17 500	12 500	12 500	
	33. Activity 1.3.3 Ongoing DNA testing	30 000	38 000	39 930	EUR / USD exchange rate change during the course of the project
	34. Activity 1.3.4 Statistical analysis	**-**	**-**	**-**	
	35. Component Total	**55 500**	**55 500**	**57 430**	
40.	**Project coordination and reporting**				
	41 Lead consultant	16 200	16 200	16 200	Additional project management required due to delays in sampling after change in project scope and delays with CITES permit issuance
	42 Project manager	19 500	12 500	19 130	
	43. Administrative support	8 400	5 400	8 862	
	43. Component Total	**44 100**	**34 100**	**45 786**	
100.	**GRAND TOTAL**	**196 100**	**196 100**	**223 716**	