



**HAL**  
open science

## Global Timber Tracking Network General sampling guide: Development of international standards and GTTN database

Nele Schmitz, Celine Blanc-Jolivet, Maria-Teresa Cervera, Manuel Chavesta, Richard C. Cronn, Victor Deklerck, Carmen Díaz-Sala, Eleanor Dormontt, Peter Gasson, David Gehl, et al.

► **To cite this version:**

Nele Schmitz, Celine Blanc-Jolivet, Maria-Teresa Cervera, Manuel Chavesta, Richard C. Cronn, et al.. Global Timber Tracking Network General sampling guide: Development of international standards and GTTN database. 2019, 10.13140/RG.2.2.26883.96806 . hal-03772051

**HAL Id: hal-03772051**

**<https://hal.inrae.fr/hal-03772051v1>**

Submitted on 7 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



GTTN

Global Timber  
Tracking Network

## General sampling guide

**Task 2:** Development of international standards and GTTN database

**Activity 2.1:** International standards

**Deliverable 2.1.1:** Reviewed GTTN guidelines on sampling of reference material

**Date:** 7 February 2019





Recommended citation:

Schmitz, N. (ed.), Blanc-Jolivet, C., Boner, M., Cervera, M.T., Chavesta, M., Cronn, R., Degen, B., Deklerck, V., Diaz-Sala, C., Dormontt, E., Ekué, M., Espinoza, E.O., Gasson, P., Gehl, D., Gehre, M., Haag, V., Hermanson, J.C., Honorio-Coronado, E., Koch, G., Lancaster, C., Lens, F., Liendo-Hoyos, E.P., Martínez-Jarquín, S., Montenegro, R., Paredes-Villanueva, K., Pastore, T., Ramananantoandro, T., Rauber-Coradin, V.T., Ravaomanalina, H., Rees, G., Sebbenn, A.M., Tysklind, N., Vlam, M., Watkinson, C., Wiemann, M. 2019. General sampling guide for timber tracking. Global Timber Tracking Network, GTTN Secretariat, European Forest Institute and Thuenen Institute.

DOI:10.13140/RG.2.2.26883.96806

The following document is an output from GTTN, which is coordinated by the European Forest Institute (EFI) and funded by the German Federal Ministry for Food and Agriculture (BMEL). The information expressed in this document are the views of the authors and do not necessarily represent those of the donor or of the European Forest Institute.

# General sampling guide for timber tracking

## How to collect reference samples for timber identification

---

**Editor:** Nele Schmitz

**Authors\*:** Céline Blanc-Jolivet<sup>1</sup>, María Teresa Cervera<sup>2</sup>, Manuel Chavesta<sup>3</sup>, Richard Cronn<sup>4</sup>, Victor Deklerck<sup>5</sup>, Carmen Diaz-Sala<sup>6</sup>, Eleanor Dormontt<sup>7</sup>, Peter Gasson<sup>8</sup>, David Gehl<sup>9</sup>, Volker Haag<sup>10</sup>, John C. Hermanson<sup>11</sup>, Eurídice Honorio Coronado<sup>12</sup>, Cady Lancaster<sup>13</sup>, Frederic Lens<sup>14</sup>, Estephanie Patricia Liendo Hoyos<sup>15</sup>, Sandra Martínez-Jarquín<sup>16</sup>, Rolando Montenegro<sup>3</sup>, Kathelyn Paredes Villanueva<sup>17,18</sup>, Tereza Pastore<sup>19</sup>, Tahiana Ramananantoandro<sup>20</sup>, Harisoa Ravaomanalina<sup>21</sup>, Alexandre Magno Sebbenn<sup>22</sup>, Niklas Tysklind<sup>23</sup>, Mart Vlam<sup>18</sup>, Charlie Watkinson<sup>24</sup>, Michael Wiemann<sup>11</sup>

**With contributions from\*:** Markus Boner, Bernd Degen<sup>1</sup>, Marius Ekué, Edgard O. Espinoza<sup>25</sup>, Matthias Gehre<sup>26</sup>, Gerald Koch<sup>10</sup>, Vera T. Rauber Coradin<sup>19</sup>, Gareth Rees<sup>27</sup>

We also thank Tom De Mil and Priwan Srisom for their photo contributions.

\*Names are listed in alphabetical order.

Edition 7 Feb. 2019

<sup>1</sup> Thünen Institute of Forest Genetics, Grosshansdorf, Germany

<sup>2</sup> Centro de Investigación Forestal, El Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CIFOR), Madrid, Spain

<sup>3</sup> Laboratorio de anatomía e identificación de maderas, Universidad Nacional Agraria la Molina, Lima, Peru

<sup>4</sup> US Forest Service Research & Development, Washington, DC, USA

<sup>5</sup> Laboratory of Wood Technology, Ghent University, Belgium

<sup>6</sup> Dept. Ciencias de la Vida, Universidad de Alcalá, Madrid, Spain

<sup>7</sup> University of Adelaide, Adelaide, Australia

<sup>8</sup> Royal Botanic Gardens, Kew, UK

<sup>9</sup> Environmental Investigation Agency, Washington, DC, USA

<sup>10</sup> Thünen Institute of Wood Research, Bergedorf, Germany

<sup>11</sup> US Forest Service Forest Products Laboratory, Madison, WI, USA

<sup>12</sup> Instituto de Investigaciones de la Amazonía Peruana (IIAP), Iquitos, Peru

<sup>13</sup> Wood Identification & Screening Center, USFS International Programs, National Forensics Laboratory, U.S. Fish & Wildlife Service, Ashland, OR, USA

<sup>14</sup> Naturalis Biodiversity Center, Leiden, The Netherlands

<sup>15</sup> Autoridad de Fiscalización y Control Social de Bosques y Tierra, Santa Cruz de la Sierra, Bolivia

<sup>16</sup> Department of Biochemistry and Biotechnology, CINVESTAV Unidad Irapuato, Irapuato, Mexico

<sup>17</sup> Universidad Autónoma Gabriel René Moreno, Santa Cruz, Bolivia

<sup>18</sup> Wageningen University & Research, Wageningen, The Netherlands

<sup>19</sup> Laboratório de Produtos Florestais, Serviço Florestal Brasileiro, Brasília, Brazil

<sup>20</sup> Mention Foresterie et Environnement, Ecole Supérieure des Sciences Agronomiques, Université d'Antananarivo, Antananarivo, Madagascar

<sup>21</sup> Mention Biologie et Ecologie Végétales, Faculté des Sciences, Université d'Antananarivo, Antananarivo, Madagascar

<sup>22</sup> Instituto Floresta de São Paulo, São Paulo, Brazil

<sup>23</sup> Institut National de Recherche Agricole, Kourou, Guyane Française

<sup>24</sup> Agroisolab UK Ltd, York, UK

<sup>25</sup> National Forensics Laboratory, US Fish & Wildlife Service, Ashland, OR, USA

<sup>26</sup> Laboratory for Stable Isotopes, Department for Isotope Biogeochemistry, Helmholtz-Centre for Environmental Research, Leipzig, Germany

<sup>27</sup> Elementar UK Ltd, Stockport, UK

## Table of Contents

|  |           |
|--|-----------|
| RATIONALE .....  | 3         |
| ABBREVIATIONS .....  | 4         |
| QUICK GUIDE.....   | 5         |
| CHECK LISTS .....  | 6         |
| <i>Checklist preparatory work</i> .....  | 6         |
| <i>Checklist fieldwork</i> .....   | 7         |
| <b>1. PREPARATORY WORK .....</b>   | <b>8</b>  |
| 1.1 CODE OF CONDUCT.....   | 8         |
| 1.2 BUDGET.....  | 8         |
| 1.3 LOCAL SUPPORT .....  | 9         |
| 1.3.1 <i>Find a local partner institute</i> .....  | 9         |
| 1.3.2 <i>Set up a local sampling team</i> .....  | 10        |
| 1.4 SAMPLING DESIGN .....  | 10        |
| 1.4.1 <i>Scientific set-up</i> .....   | 10        |
| 1.4.2 <i>Practical set-up</i> .....  | 15        |
| <b>2. FIELD WORK .....</b>   | <b>17</b> |
| 2.1 SPECIES IDENTIFICATION IN THE FIELD.....   | 17        |
| 2.2 THE SAMPLE RECORD: COLLECTING TREE & SITE INFORMATION .....  | 18        |
| 2.3 COLLECTING SAMPLES.....  | 20        |
| 2.3.1 <i>Overview of reference material to be collected enabling species/origin identification by all methods</i><br>..... | 21        |
| 2.3.2 <i>How to collect leaves, fruits and flowers &gt; herbarium specimen and DNA analysis</i> .....                      | 22        |
| 2.3.3 <i>How to collect wood samples &gt; all timber identification methods</i> .....                                      | 23        |
| <b>3. TRANSPORT &amp; STORAGE OF SAMPLES AND DATA.....</b>   | <b>25</b> |
| 3.1 FOREST-TO-LAB SAMPLE CHAIN & SAMPLE QUALITY .....  | 25        |
| 3.2 SAMPLE STORAGE IN THE FIELD .....  | 25        |
| 3.3 SAMPLE TRANSPORT.....  | 28        |
| 3.4 LONG TERM SAMPLE STORAGE.....  | 28        |
| <b>4. REFERENCES .....</b>   | <b>29</b> |
| <b>5. APPENDICES .....</b>   | <b>32</b> |
| APPENDIX 1: ILLUSTRATIONS TO THE SAMPLING GUIDE.....   | 32        |
| APPENDIX 2: SAMPLING MATERIAL & EQUIPMENT .....  | 39        |
| APPENDIX 3: EXAMPLES OF FORMS TO COLLECT FIELD DATA .....  | 40        |

## Rationale

This is a guide for the collection of *reference samples* of trees to enable the **identification of species and/or geographical origin of woody material**. It is an update of the sampling section of the [GTTN standards and guidelines](#) (Ekué 2014) and builds further on a discussion initiated during a workshop held in Hamburg at the Thünen Institute for Wood Research in 2014. If you are looking for support on how to collect *test samples*, see the UNODC guide (UNODC 2016).

To enable the implementation of the different laws regulating the trade in illegal wood, **reference databases** for various timber tracking tools are urgently needed for at least the most traded and endangered tree species. The [Global Timber Tracking Network](#) (GTTN) is building a central database where not only the reference data can be stored but which will also function as a sample locator. Having a common sampling guide will facilitate meaningful exchange of samples.

In addition, to optimise the use of wood/wood product identification (taxonomic identity or geographic origin) in support of law enforcement, the guide anticipates upcoming developments to combine (Paredes Villanueva 2018) different timber identification methods (Dormontt *et al.* 2015, Lowe *et al.* 2016) such as **wood anatomy** (Koch and Schmitt 2015, Helmling *et al.* 2018), **DNA-based methods** (Jolivet and Degen 2012, Blanc-Jolivet *et al.* 2018, Chaves *et al.* 2018), **stable isotopes** (Paredes-Villanueva *et al.* in preparation, Vlam *et al.* 2018), **DART TOFMS** (Lancaster and Espinoza 2012, Espinoza *et al.* 2015, Deklerck *et al.* 2017, Paredes-Villanueva *et al.* 2018) and **NIRS** (Pastore *et al.* 2011, Bergo *et al.* 2016, Snel *et al.* 2018). This sampling guide is written to make sharing of samples between researchers specialised in different timber tracking methods possible, as samples should ideally come from the same location in the tree, from the same individual and from well-identified trees when combining methods.

**This guide is intended for scientists**, to provide all the information needed to get the most out of sampling campaigns for timber identification purposes. This information should allow setting up a sampling protocol adapted to the specific goal of the research project, the conditions of the sampling area and the background of the people who will do the sampling. Note that this guide is to collect reference samples and hence relatively high amounts of samples from different individuals are needed to take the variability of a species into account. Once reference data have been developed for a tree species for one or more identification methods, however, only one sample of an unidentified wooden object is often sufficient to determine its identity.

## Abbreviations

|                    |   |
|--------------------|---|
| <b>AAC</b>         | Assiettes Annuelles de Coupe (Annual Cutting Area)                              |
| <b>°C</b>          | Degrees Celsius   |
| <b>Ca.</b>         | Circa   |
| <b>CITES</b>       | Convention on International Trade in Endangered Species of wild fauna and flora |
| <b>∅</b>           | Diameter  |
| <b>DART TOFMS</b>  | Direct Analysis in Real Time Time-of-Flight Mass Spectrometry                   |
| <b>DBH</b>         | Diameter at Breast Height   |
| <b>DF10</b>        | Document specifying the timbers extracted from the forest                       |
| <b>DNA</b>         | DeoxyriboNucleic Acid   |
| <b><i>e.g.</i></b> | for example   |
| <b>EUTR</b>        | EUropean Timber Regulation  |
| <b>GPS</b>         | Global Positioning System   |
| <b>GTTN</b>        | Global Timber Tracking Network  |
| <b>ID</b>          | Identification  |
| <b>Min.</b>        | Minimum   |
| <b>NGO</b>         | Non-Governmental Organisation   |
| <b>NIRS</b>        | Near InfraRed Spectroscopy  |
| <b>Pvc</b>         | Polyvinyl chloride  |
| <b>RH</b>          | Relative Humidity   |
| <b>Sample ID</b>   | Sample IDentity   |
| <b>UNODC</b>       | United Nations Office on Drugs and Crime  |

# Quick guide

## The ideal reference sample collection for timber identification

### Preparation

➤ p. 8



### Local support & Code of conduct

- Identify local expertise and knowledge to build a strong sampling team
- Follow local up to international regulations

### Budget



- Often underestimated
- Plan well!

### Sampling design



- Scientific: review on site & species
- Practical: time, tools, transport

### Fieldwork

➤ p. 17



➤ p. 12

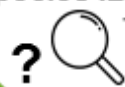


➤ p. 32



➤ p. 39

### 1. Species ID in the field



### 2. Collecting tree & Site data



➤ p. 18

### 3. Collecting samples



➤ p. 20

### 4. species ID in the lab from unknown samples = GOAL



For DNA analysis:  
Option A > leaves  
Option B > cambium

3 cm Ø punch  
thin bark



thick bark

Herbarium sample



11 cm long, 20 mm Ø increment core

for wood anatomical analysis



If recently felled trees, no need to core

3 x 25 cm long, 5 mm Ø increment cores

Min. 5 cm of heartwood for DART TOFMS/NIRS analysis

Min. 8 growth years or 10 cm for stable isotope analysis

Bark for future research



### Storage & Transport



➤ p. 25

### Assuring sample quality

- Forest to lab sample chain
- Unique & consistent labels
- Correct storage in the field and long term



### Prevent:

- moulding
- bacterial or insect damage
- DNA degradation
- contamination
- tissue shrinkage (for wood anatomy)
- Verify and deposit herbarium vouchers and duplicate wood samples at public reference collections



## Check lists

### Checklist preparatory work

#### **Before all else:**

1. Did I consider costs for permits, transport of the sampling team, transport of samples back to the lab, payment of sampling team, accommodation and subsistence, sampling material and equipment? ▶ [1.1-1.2](#)
2. Did I get permits to do research in the different sampling sites, to collect samples and to export and import them? ▶ [1.1-1.2](#)
3. Did I explore the available local knowledge and expertise and find local partners to build a local sampling team? ▶ [1.3](#)

#### **Specifying the aim of the mission:**

4. Did I clarify the research question of the sampling campaign? ▶ [1.4.1](#)
5. Did I do a scientific literature review on the species and sites that will be sampled to collect all basic information required? ▶ [1.4.1](#)

#### **To decide beforehand:**

6. Did I decide on how to select sites and trees within sites? ▶ [Table 1, 1.4.1](#)
7. Did I decide on the amount of material that will be sampled (based on budget and essential quantities)? ▶ [Table 1, 2.3.1](#)
8. Did I decide on the site and tree data that will be collected and how? ▶ [2.2, 3.1, appendix 3](#)
9. Did I decide on how samples will be stored in the field, during transport and when back at the lab? ▶ [2.3.2-3, 3.2-3.4](#)
10. Did I decide on the material and equipment to be used? ▶ [Table 1, Appendix 2](#)
11. Did I decide on a labelling code? ▶ [1.4.2](#)
12. Did I decide on all other practicalities for the field work? ▶ [1.4.2](#)

## Checklist fieldwork

### **Packing:**

1. Do I have all required material and equipment for the amount of samples that I want to sample? ▶ [Appendix 2](#)
2. Do I know how to label or is all material pre-labelled? ▶ [1.4.2](#)
3. Do I have what is needed to identify the tree species of interest in the field? ▶ [2.1](#)

### **At the field site:**

4. Start recording the field trip in your notebook/on your template form ▶ [2.2](#), [Appendix 3](#)
5. Collect site information ▶ [2.2](#)
6. Collect herbarium material and leaf samples ▶ [2.3.2](#)
7. Collect wood samples ▶ [2.3.3](#)
8. Collect and record all tree info ▶ [2.2](#)

### **At the field station/camping area:**

1. Dry wood cores/samples and change humid silica for fresh one ▶ [3.2](#)
2. Assemble herbarium specimens if not done yet, change humid newspapers for dry ones or add alcohol if drying the herbarium material later ▶ [3.2](#)
3. Check, complete and organise field notes where needed, digitise if already possible ▶ [3.1](#)

# 1. Preparatory work

## 1.1 Code of Conduct

The first principle that has to be considered is the sovereign rights of states over their forest resources. Collection, transport, processing, management and storage of material from forest trees have to be performed in accordance with the **national and local regulations** (ask for information from *e.g.* your local partner(s), forester, concession/land owner, park authorities). In addition, the sampling campaign should be in line with the existing **regional regulations** such as the EUTR, the US Lacey Act and the Australia Illegal Logging Prohibition Act (see *e.g.* [here](#) for more information) and with **international regulations** such as CITES and the [Nagoya protocol](#) (an explanatory guide can be found [here](#)). For information about the requirements concerning CITES listed species you can contact [national CITES authorities](#).

Accordingly, research **permits** for field collection, Material Transfer Agreements or other appropriate documentation must be requested well in advance to ensure the correct collection, transport and management of the forest tree material harvested and stored as reference samples. In addition, the **community/ies living in the area of sampling need to be informed** on the sampling campaign (as some might for example be worried the bore holes will damage the trees).

## 1.2 Budget

Sampling costs are often underestimated. Before planning your sampling campaign contact the [GTTN network](#) and the GTTN followers via the [ResearchGate project page](#) to find out if you can team up with others interested in sampling in the region to make the trip more cost-efficient. It is advisable to account for the following expenses when budgeting:

- **Any fees related to getting permission** and support from both national and local authorities for the planned sampling and for transportation of the samples from the field to the lab.
- **Transportation to the different sampling sites:** costs will be related to accessibility. Inform yourself on the means and duration of transportation required to reach the different sampling sites and the related costs (vehicle, driver, fuel costs).

- **Transportation and/or shipping of the samples** to the laboratory, including potentially required phytosanitary certificates.
- **Payment for assistance** by people knowing (i) the area and (ii) the tree species during the entire journey to and in the forest. Consider sampling efficiencies as low as 10 trees per day for tree species with low densities.
- **Accommodation and subsistence.**
- **Sampling material and equipment** (see [Appendix 2](#)).

**TIP:** If you will need a **car** and you have the choice, pick one with a functioning cigarette lighter (accessory power outlet). This will enable you to charge batteries (for GPS, electric increment borer, laser meter, camera, computer) in the car when needed.

**TIP:** To be able **to estimate the sampling work that can be done in one day** if samples are taken as described in *§2.3 Collecting samples*, it is advisable to do field tests with the sampling team. The duration of a sampling campaign will depend on variables such as: species density, available equipment (*e.g.* mechanical or hand borer), time needed to get to the canopy (to collect leaves), chosen intensity of herbarium specimen collection, number of timber identification methods material is collected for, experience of the field team.

## 1.3 Local support

### 1.3.1 Find a local partner institute

**TIP:** It is recommended to include local partners from the project design onwards to make sure that the project interests both sides and the local partner does not just serve as a collector.

Identification of **local partners** (universities, research institutes, NGOs, companies, ...) which already have expertise and/or interest in timber identification techniques and/or have some infrastructure, material and trained personnel.

The local partner will be able to advise on a **local botanist/(para)taxonomist, an experienced driver and a field guide**, who know the area and its species as well as its dangers. They are an indispensable part of the field team as guides in the forest to find the targeted trees, facilitate interaction with local communities and to reduce the risk of attacks from animals or hostile people (*e.g.* illegal loggers, miners).

Get advice from your local partner on how to get the required **permit(s) to collect and export** samples and who should be contacted before arriving at the different sites you want to sample (*e.g.* community leaders, officials, company personnel). Check if some physical samples can be stored in a local herbarium (see [Index](#))

[Herbariorum](#)) and/or xylarium (see [Index Xylariorum](#)) and taxonomically identified by specialists (start with checking the [GTTN network](#) to find contacts).

Identify **local students** who are working or might work on the species of interest and might be interested in co-authoring the research papers and/or to participate in the expedition.

### 1.3.2 Set up a local sampling team

Create a base of trust both with the local community and within the sampling team before starting the sampling campaign and make sure everyone knows the role and responsibility of each other. In case the principal investigator cannot participate for the full length of the sampling campaign, his/her presence at the start of the sampling is necessary to train the people who will do the sampling and adapt the sampling protocol if necessary.

- Use the local knowledge on species identity, variability, density and sites of occurrence provided by botanists, ecologists, local guides and collaborators.
- At least one person should be scientifically trained and understand the reasoning behind the sampling design and be responsible for oversight of the sample collection accordingly, for note taking and for correct GPS reading.
- At least one person should be technically trained and responsible for sample collection according to protocol and maintenance of equipment.
- Depending on the conditions additional expertise might be necessary: a person that can use a gun, a driver used to the terrain that will be sampled, a tree climber, a person trained in using a sling shot.

## **1.4 Sampling design**

### 1.4.1 Scientific set-up

To be able to set-up the sampling design a **scientific literature review** and general information search should be undertaken to collect as much information as possible on the species and geographic locations of interest. The thoroughness of the review on the geographic location(s) will depend on the **goal of the sampling**, species or origin identification and the required resolution of the origin identification. [Table 1](#) gives an overview of the reference material that needs to be collected to allow species or origin identification using the different tracking methods.

**Information that should be collected** (where applicable for the specific wood identification goal of the sampling):

- **To decide on where to go sampling** (which countries and locations)
  - samples already available (check [GTTN's reference database](#))
  - species distribution (focus on natural occurrence not on political borders)
  - intraspecific species diversity (genetic variation, which might also influence anatomical and chemical properties)
  - species abundance (a minimum of 20 individual trees per species of interest should be available for sampling in an area of 1 km<sup>2\*</sup>)
  - spatial distribution of species in forest concession (forest inventory map)
  - environmental variation (include as much as possible)
  - chance of getting a permit to sample at the sites of interest
  - accessibility and feasibility (infrastructure)
  - safety (political situation, terrain)
  - relevance for the timber trade (areas where legal and/or illegal harvesting is currently happening, or where it is projected to happen)
  - risk of endangering the species population<sup>†</sup>
  - possibility to partner with a concession holder and to sample during or shortly after logging (within one week at most and with trees still lying at the felling site, to guarantee fresh wood and leaves and the leaves' origin)
- **To decide on when to go sampling**, balancing the ease to identify species (flowers or fruits available), the ease to do field work (dry season) and minimising tree injury by coring (faster compartmentalisation of the wound in the growing season<sup>‡</sup>)
  - species phenology (months of leaf flushing, flowering, fruiting)
  - climatic conditions (see §1.4.2 practical set-up)
- **To decide on what to sample**
  - taxonomically closely-related species or cryptic species
  - trunk diameter found in trade and diameter at which the species starts forming heartwood in the location of interest
- **To anticipate potential identification issues**
  - potential association with rhizobia (can influence isotope profile)
  - seed/tree source of species in the forest concession

---

\* For heavily harvested species where this might be impossible, select sites with the highest tree density available.

<sup>†</sup> *E.g. Neo et al. (2017)*

<sup>‡</sup> *Grissino-Mayer (2003), Tsen et al. (2016)*

**Table 1.** Overview of the essential and ideal amount of reference material that needs to be collected for species or geographic origin determination of wood via the currently available techniques.

| Design questions                            | Wood anatomy   | DNA  | Multi-element stable isotopes   | DART-TOFMS  | NIRS  |
|---|--|--|---|---|---|
| <b>For all questions</b>                    |  |  |   |   |   |
| <b>general requirements</b>                 | Sample all material (leaves, wood) from mature trees (DBH larger than 20 cm), at breast height or 30 cm above buttresses <sup>I</sup> , where no stains or damage from bacteria, fungi or insects are visible and from trees growing in as varied environments as possible (soil type, altitude, exposure, fresh water access, ...). Assure an even distribution of the number of individuals among sampling sites, with a preference for more sampling sites with fewer trees per location. |  |   |   |   |
| <b>type of material</b>                     | Sap- and/or heartwood  | Leaves, needles, buds and/or cambium   | Sap- or heartwood or both   | Heartwood <sup>II</sup>   |   |
| <b>amount of material per sample</b>        | Block of 1 cm <sup>3</sup> or a 20 mm diameter core or (ideal) 1 x 7 x 11 cm wood piece <sup>III</sup>   | 10 cm <sup>2</sup> of leaves/needles/buds or 3 cm diameter punch of cambium layer or (but less ideal) 1 cm <sup>3</sup> of sapwood               | Min. 8 growth years or <i>ca.</i> 10 cm of a 5 mm diameter core (5 g of wood in shavings) | A small core (3-5 slivers, 10-20 optimal, with a sliver being of fingernail size is enough) | Blocks <sup>IV</sup> of min. 2 cm <sup>2</sup> in tangential or radial longitudinal direction |
| <b>replicates<sup>V</sup></b>               | 1 per tree <sup>VI</sup>   | 3 per tree   | 3 per tree  | 1 per tree  | 3 per tree  |
| <b>preferred equipment</b>                  | Increment borer, chisel and hammer, saw  | Telescopic scissors or sharpened hook, sling shot, puncher and mallet  | Increment borer <sup>VII</sup> (manual or mechanical)                                     |   |   |
| <b>For species identification</b>           |  |  |   |   |   |
| <b>botanical material</b>                   | 1 herbarium specimen (branch with leaves, fruits and/or flowers and optional a piece of bark) per tree   |  |   |   |   |
| <b>nr. of trees &amp; sites (essential)</b> | 5 trees or 5 trees per site if environment changes   | 50 trees over the whole species range  | <i>not possible with this method</i>  | 15 trees  | 20 trees  |
| <b>outgroup (ideal)</b>                     | At least 5 trees should be collected from each species that could be confused with the species of interest (same genus).   |  |   |   |   |
| <b>nr. of trees &amp; sites (ideal)</b>     | 20 trees over the whole species range (for machine vision)   | 10 trees per sampling site with a total min. of 50 if covering the whole species range. More sampling sites are better than more trees per site. | <i>not possible with this method</i>  | 20 trees  | 30 trees  |

**Table 1. (continued)**

| Design questions                                  | Wood anatomy   | DNA   | Multi-element stable isotopes   | DART-TOFMS  | NIRS  |
|---|--|---|---|---|---|
| <b>For origin tracking to a region or country</b> |  |   |   |   |   |
| botanical material                                | Pictures of trunk, leaves, and if possible fruits and/or flowers per tree and 1 herbarium specimen per site <sup>viii</sup> . If one tree is difficult to identify, then a herbarium specimen should be taken. |   |   |   |   |
| nr. of trees & sites (essential)                  | <i>not possible with this method</i>   | 20 trees per sampling site  | 5 trees per sampling site   | 50 trees <sup>ix</sup> in total for 1 region/country    | 50 trees <sup>ix</sup> in total for 1 region/country    |
| nr. of trees & sites (ideal)                      |  | 30 trees <sup>x</sup> (at least 200 m apart <sup>xi</sup> ) per sampling site (at least 100 km apart) with a total of 1000 trees and sites covering the entire species range and all different environmental conditions | Each time 10 trees per sampling site and sampling sites covering entire species range | 100 trees, sampling sites covering entire species range | 100 trees, sampling sites covering entire species range |
| <b>For origin tracking to a concession</b>        |  |   |   |   |   |
| botanical material                                | Pictures of trunk, leaves, and if possible fruits and/or flowers per tree and 1 herbarium specimen per site <sup>xii</sup> .   |   |   |   |   |
| nr. of trees & sites (essential)                  | <i>not possible with this method</i>   | Per focus concession 200 trees at least 50 m apart <sup>xi</sup> (5 x 40 trees in the annual logging plot and 4 other well-distributed areas) and from each neighbouring concession 50 trees (can be along a transect)  | 50 trees per concession and from each neighbouring concession 25 trees                | 50 trees <sup>ix</sup>                                  | 50 trees <sup>ix</sup>                                  |
| nr. of trees & sites (ideal)                      |  | Sample size depends on concession size and distance to neighbour concessions  | Depending on the climatic or environmental variations in a sample site                | 100 trees   | 100 trees   |
| <b>For origin tracking to an individual tree</b>  |  |   |   |   |   |
| botanical material                                | 1 herbarium specimen (branch with leaves, fruits and/or flowers) per tree <sup>xiii</sup>  |   |   |   |   |
| nr. of trees & sites (essential)                  | <i>not possible with this method</i>   | all trees which should be felled according to management plan   | <i>not possible with this method</i>  | <i>not possible with this method</i>                    | <i>not possible with this method</i>                    |



---

<sup>I</sup> Wood characteristics change from roots to canopy and it is hence advisable to standardise the height of sample collection. Also near buttresses (and any other imperfections) wood characteristics are deviant.

<sup>II</sup> Heartwood in slivers, blocks, or sawdust is required for chemical analysis by DART TOFMS and NIRS. Heartwood has a higher content of extractives than sapwood which allows easier discrimination between species. In addition, sapwood contains sugars that confuse the spectra for identification. Different species of trees have varying degrees of depth at which heartwood forms so care should be taken to clearly identify and collect the heartwood.

<sup>III</sup> Only possible from already felled trees.

<sup>IV</sup> Also powder of 4 mm granulometry can be used to obtain a NIRS spectrum. Only on wood pieces, however, can the method be used in the field. Besides, during the milling process special care should be taken to not affect the chemical components in the wood.

<sup>V</sup> Replicates might be needed to collect enough material and to account for intra-tree variation.

<sup>VI</sup> For machine vision it is however useful to sample from different positions in the tree to include as much intra-tree and intra-specific variation as possible (but while sampling only mature wood).

<sup>VII</sup> Advice and tips for using an increment borer can be found in Grissino-Mayer (2003) and examples of mechanical borers are described at <http://www.smartborer.com> (Kagawa and Fujiwara, 2018) and in Krottenthaler *et al.* (2015).

<sup>VIII</sup> Ideally, each reference sample should be connected to a herbarium specimen (preferably branch with leaves, flowers and/or fruits) deposited in a public herbarium. However, this is not always possible (*e.g.* when sampling 1000 trees for provenance determination).

<sup>IX</sup> Origin tracking with DART and NIRS is currently under development. The required number of trees might thus lower in future.

<sup>X</sup> Double the number of individuals if congeneric species may confound species identification.

<sup>XI</sup> This condition is lifted when tree density is too low to otherwise reach the minimum sample size.

<sup>XII</sup> Also in concessions misidentifications can happen.

<sup>XIII</sup> Even here herbarium specimens are essential because (1) many journals won't accept wood identification papers that don't reference herbarium specimens and (2) when the material would ever be used in a court case, the absence of herbarium specimens would harm the case.

### 1.4.2 Practical set-up

Take the necessary **personal precautions**. Get an update on the latest political situation at the sampling sites and check if specific health precautions should be taken, *e.g.* required vaccinations.

Check the climatic conditions of the region to identify an appropriate **sampling period**. In tropical areas avoid rainy seasons and in mountainous/continental regions avoid cold months, as strong rain or heavy snowfall may reduce or impair the accessibility of sampling sites and thus increase the costs of sampling.

Decide on the **means of transport** to the different sampling sites (plane, boat, 4x4/car, taxi, bike, canoe, by foot or combination) and make the necessary arrangements.

Decide on the **sampling material and equipment** (see Table 1 and Appendix 2) and prepare. Provide also a spare set as replacement may not be possible at local markets.

Decide on the **amount of material** you will collect (see Table 1 for the essential and ideal amounts). Consider informing the GTTN members via the [website](#) or the [ResearchGate project page](#) as there might be interest in joining forces and hence to sample more material that can then benefit more researchers at once. At least 1 herbarium specimen is needed per tree or site (see Table 1) but preferably duplicates are collected to be sent to other herbaria along with duplicate wood specimens in order to safeguard the collection material.

Set up a robust and consistent **labelling strategy**. All reference material (wood, cambium, bark, leaf, flower, fruit) collected from the same tree should be labelled with the date of collection and the same, unique sample code that should reference back to a sample record identifying at least:

- the location
- the species<sup>§</sup>
- the tree
- the position around the tree's circumference
- the radial position in the tree where the sample was taken

Check **regulations on import** of wood and botanical material.

---

<sup>§</sup> It is important to notice that the species can only be confirmed after accurate identification (by a specialized taxonomist). The intended species to be collected in the field is, therefore, not necessarily the actually collected species. The final ID might hence differ from the field ID.

Decide on the **wood collection and herbarium** you will use to store your wood samples and herbarium specimens (check the [Index Xylariorum](#) and the [Index Herbariorum](#)). Preferably, send your herbarium specimen and wood sample to the same institute since it is not desirable that these two specimens are separated from each other. If not possible, make sure the two institutions have the associated sample data.

**TIP:** To save work in the field (where conditions might also be less favourable to be writing), sets of **pre-labelled bags/vials** can be prepared beforehand with different sets per tree, per site, per species, ... that can be grouped in a bigger bag or container, or strung along a small cord. In this way, it also immediately becomes clear when you forget to take a sample as then one bag will stay empty. Make sure to label with water resistant and alcohol resistant marker pens.

**TIP:** Avoid editing the unique sample code of the samples. If you decide to do so, remember to apply the change to all collected material (herbarium specimen, leaves, cambium, wood, ...).

## 2. Field work

### 2.1 Species identification in the field

Correct species identification is essential when collecting wood samples for a reference database (to identify species or geographical origin of wooden material) as mentioned in the UNODC guide (UNODC 2016):

***Accurate taxonomy is critical. Reference material should be collected by (or in collaboration with) experts on field identification of the taxa in question. Where possible, trees already taxonomically verified*** [by having collected an herbarium specimen so that the taxonomic identity of the wood sample can be verified by other researchers] *can be utilized, such as those that are part of permanent study plots or botanic gardens (although use of botanical garden specimens should be considered carefully as misidentifications can occur, and should be avoided altogether for anatomical or provenance research where the garden is outside of the taxon's natural range). When collecting from standing trees that have not yet been taxonomically identified, **herbarium voucher\* specimens should be collected to allow post-hoc confirmation** of identity by herbarium taxonomists* [How to collect herbarium specimens is described in §2.3.1].

It is highly advisable to engage a local botanist/(para)taxonomist who can help with **identifying the species of interest in the field**. Tree species can be recognised by tree morphology and by the characteristics of their leaves, fruits, flowers (see §1.4.1 > consult relevant literature for time of flowering/fruitletting) and bark. By cutting the bark with a machete the wood's colour and smell can also assist in identification. A printed picture of a herbarium specimen of the species could be taken along in the field.

In the case that it is not possible to identify the tree with 100 % certainty in the field, it is still worth to take the sample along together with a herbarium specimen (make one for at least one sample per species and per site) and a note of the uncertainty of the identification in the sample record. The herbarium specimen can be examined at the herbarium and in many cases assigned to the correct species.

***Where it is impractical to collect vouchers for all individuals*** (e.g. where foliage is high in a dense canopy or when deciduous trees are bare), identification should be confirmed through other means by checking that samples cluster with taxonomically

---

\* A herbarium specimen is sometimes called a voucher.

*verified individuals, such as through various genetic or chemical profiling approaches* (UNODC 2016).

## 2.2 The sample record: collecting tree & site information

For each tree sampled, information on the tree and the site should be collected to make the sample suitable for species and/or origin identification. This is especially important if the samples will be shared with other researchers, will be analysed via different timber identification methods or if there were unexpected changes to the sampling plan. In addition, this information is needed to prepare the herbarium specimen label. Below you can find a list of all data that are recommended to be recorded in a notebook or on a template, which can be developed for convenience in the field (For examples see [Appendix 3](#)).

### For each sampling campaign:

- Collection season (this can influence fruit/flower size and hence species identification)
- Names of the members of the sampling team

### For each sampling site:

- Collection locality:
  - Political divisions (*e.g.* country, province, region, district, commune, locality)
  - Concession (if applicable)
  - Habitat description (*e.g.* forest type, altitude, list of other characteristic taxa, other site characteristics)
  - Climatic zone
- Name of collector
- Name (if different from collector) and species identification qualifications of the person who identified the species
- Tree characteristics used to identify each species

### For each tree sampled:

- Collection date
- GPS coordinates as latitude and longitude (in decimal degrees with at least four decimals) and using WGS84 as datum
- Unique labelling codes of all<sup>†</sup> samples taken (see §1.4.2)

---

<sup>†</sup> This applies to all types of tree material (leaves, bark, cambium, wood).

- ☑ Scientific species name, or if not possible the local species name, and a note if identification is uncertain
- ☑ Specimen description (attributes which cannot be observed from the samples collected):
  - Tree circumference or diameter at breast height (cm)
  - Tree height (m) (estimation using clinometer and laser meter/tape measure or a hypsometer)
  - Position in the canopy (*e.g.* lower, middle, upper)
  - Samples taken from a standing or recently felled tree (and inventory number if sampled from a concession)
  - What other material was collected (note down which tissue types were sampled for each tree)
- ☑ Photos (advisable) with an object of known size (*e.g.* ruler, pen, coin) as scale bar and with the individual sample ID to later verify species identity and to help your memory in case any peculiarities pop-up of:
  - Tree in its environment
  - General shape of the tree (incl. any buttress roots)
  - Tree with extraction site visible
  - Bark and other distinctive elements (close-ups of flowers, fruits, leaves)
  - Collected material when still fresh (leaves, fruits, flowers, wood)
  - GPS device with coordinates showing and sampled tree in background, or use a sampling app that records the current location when you take the photo of the tree

**TIP:** It is advised to **tag each sampled tree** with the unique sample code (*e.g.* Fig. 1a). It can help prevent sampling the same tree twice and would allow repeat visitation of that tree (GPS coordinates are not always exact enough to get to an individual tree trunk).

**TIP:** If you have a smart phone in the field, instead of or in addition to taking GPS coordinates, you can also use a **GPS Logger app**. Alternatively, geo-tagged photos can be taken of the sample bag with the sample visible inside and the label visible. Make sure that time and date are accurate on cell phone and that location tagging is active<sup>‡</sup>. Take your precautions to avoid running out of battery.

---

<sup>‡</sup> Be aware that location data accuracy comes down to an interplay of several factors, including signal source (GPS signals, Wi-Fi, and cell tower triangulation), environment (area density, skyline view, and indoor or outdoor location), and personal use (location data access enabled, type of mobile app used, and operating system usage) [[Ref.](#)].

**TIP:** Combining the above two tips, a **mobile phone app** can be developed where sample and site information as well as pictures can be put into a database directly in the field and uploaded into the cloud as soon as networks are available.

## 2.3 Collecting samples

① In Appendix 1-3, you can find (1) illustrations of the sampling procedure described below, (2) a list of the material and equipment needed for the sampling, and (3) examples of template field forms to record the sampling.

① In Table 1, the type and amount of material, the number of trees and the number of sites to be sampled are given. Here we describe how the material should be taken and we give an overview of the material to be collected when sampling immediately for different identification methods at once.

① In chapter 3.2, more extensive information on sample storage is given.

### BOX 1. SILICA GEL-PRACTICAL INFORMATION

**Silica gel?** We recommend the use of silica gel\* since it is a good desiccant, is lightweight and it has a humidity colour indication but if not possible you could use rice husk† (Emdadi *et al.* 2017). Since it doesn't change colour, test a range of samples to get to know how fast you have to replace it. Put the rice husk for example in a tea bag inside a ziplock bag with the sample to avoid contact with the desiccant.

**How much silica gel is needed?** As a rule of thumb a 1:10 ratio of sample to silica by weight is advised. The silica is changed until the sample is fully dried and the silica doesn't change colour anymore.

**What do we mean with contamination?** In this guide it is regularly mentioned to watch out for contamination. With this we mean contamination with DNA (which would affect DNA analyses) or with chemical compounds (which would influence stable isotopes, DART and NIRS analyses). Therefore, if you want to dry and reuse silica gel beads, they should be put in for example tea bags closed with a piece of thread to avoid contaminating samples. At the same time, the self-made silica bags will facilitate exchange with bags containing fresh silica.

---

\* We recommend use of the larger (2-4 mm beads) orange/green silica gel crystals. The indicator dye cobalt chloride used in the blue/pink ones has been classified as a carcinogen in Europe. Methyl violet is offered as a safer alternative. The dust size gel is impossible not to inhale when you handle it [from: <http://plantarum.ca/botany/silica/>].

† Rice grains or salt is not recommended as their water absorption capacity is not high enough for fast desiccation and hence to prevent DNA degradation and moulding (affecting the chemical analyses).

### 2.3.1 Overview of reference material to be collected enabling species/origin identification by all methods

**Reminder:** the following instructions are to fulfil the minimum requirements for the different timber identification methods and hence to make method combinations possible. Method-specific instructions can be found in [Table 1](#).

- **Herbarium specimen:** 1 branch with mature leaves, flowers and/or fruits per tree (for species identification) or per site together with 1 set of pictures of leaves, flowers and/or fruits per tree (for origin identification)<sup>‡</sup>. For compound leaves, make sure to sample the entire compound leaf and not just a fraction.
- **Material for DNA analysis:** leaves or needles (or leaf buds if leaves didn't flush yet) with a minimum combined surface area of 10 cm<sup>2</sup>. If leaves could not be collected or as a safeguard, three 3 cm diameter punches of cambium or, less ideal but possible, 1 cm<sup>3</sup> of sapwood.
- **Material for stable isotope analysis:** one but ideally three 20 cm long and 5 mm diameter (pieces of) wood cores providing 5 g of wood (for trees of very light wood density more or longer cores might hence have to be taken).
- **Material for DART TOFMS and NIRS analysis:** 5 cm of the innermost parts of one but ideally three wood cores of 25 cm long and 5 mm diameter (the required depth of coring will depend on heartwood formation in the species and locations of interest)
- **Material for potential future identification methods:** keep the bark of the above mentioned punches and cores.
- **Material for wood anatomy:** 5 mm diameter cores are not suitable as reference material<sup>§</sup>. An extra wood sample needs to be taken at breast height of at least 1 cm<sup>3</sup>. This will guarantee that good micro-sections can be cut along all planes (even by less experienced wood anatomists), that there is a big enough surface area to analyse anatomical variations, and to allow digital image analysis.

---

<sup>‡</sup> Ideally, 2 herbarium specimens are collected so that one can remain in the herbarium and the other can be send to a taxonomist (outside of the herbarium) if necessary. If absolutely impossible to collect leaves, they can be looked at with binoculars to make a determination.

<sup>§</sup> Only when the core goes straight to the pith, sections can be made along all planes using the full size of the core. As test material, on the contrary, samples can be as small as a sliver to be analysed by wood anatomy.



### 2.3.2 How to collect leaves, fruits and flowers > herbarium specimen and DNA analysis

- From the exact tree that will be sampled for wood (*i.e.*, a mature tree): take two branches with multiple mature leaves or at least 1 mature compound leaf (Be sure the compound leaf contains all the leaflets and the whole rachis.). The branch should be big enough to show the distribution of the leaves in the branch. If available the branch should also contain flowers and/or fruits. Don't collect already fallen leaves from the ground, or from a nearby sapling.
- Put one of the branches showing at least front and back side of a leaf and if available a flower or fruit between newspapers in a botanical press for the herbarium specimen. This step can also be delayed to the end of the day when back at the field station. Label branches in the field (*e.g.* with paper tags) and transport them in big plastic bags to the field station. Press and dry samples as soon as possible. When the climate is humid and when staying in the field for more than two days, preserve the herbarium specimens in a plastic bag wetted with 50 % alcohol (and dry in an oven later) (Fig. 3e-f).
- When dealing with large fruits or fleshy flowers put them in teabags inside ziplock bags with a silica gel bag.
- The leaves of this second twig are stored for DNA analysis in a tea bag that is put inside a ziplock bag together with a silica gel bag. This is to avoid losing the leaf material as it may break into small pieces.
- Make sure all material collected is labelled.

**TIP:** More information on collecting and preparing **high quality botanical specimens** is available from [Missouri Botanical Garden](#) (Liesner, accessed 24 April 2018) or [Royal Botanic Gardens Kew](#) (Jennings *et al.* 2018).

**TIP: If collection of botanical samples is not possible**, take a photo that shows the tree characteristics. Include a ruler or a scale bar to give an idea of the size of leaves, fruits or flowers. Never collect leaves from young trees or sprouts as morphological characteristics may differ to those expressed in adult trees.

**TIP: To fasten desiccation**, the leaves taken for later DNA analysis (not the ones for the herbarium!) can be ruptured into small pieces using clean scissors (clean with alcohol for each tree). The pieces are then put in tea filters in the ziplock bags with a silica gel bag.

### 2.3.3 How to collect wood samples > all timber identification methods

#### *From standing trees*

From the same tree as you just sampled leaves, fruits and/or flowers:

- At breast height, take at least one but ideally three (see Table 1) **25 cm long 5 mm diameter wood cores** per tree at different positions around the stem's circumference. Clean the increment borer and extractor with ethanol (50-70 %) each time before sampling a new tree. When the species has a very hard outer bark, remove\* only this very outermost, hard part of the bark to facilitate coring but keep the rest of the bark (for potential future research). Store the cores in paper straws (📄 storage essentials for DART analysis†) for transport indicating orientation (pith/bark side) and closing both sides of the straws by turning. Once back at the field station/camping area dry the wood inside the paper straws by keeping them 20-30 cm above a small fire (Fig. 5d). Alternatively, plastic straws can be used, which can be closed using a lighter. However, special care should then be taken to prevent mould. At the end of the day, cores should be taken immediately out of the straws for air drying in a place with a good air flow‡. Once dry and if already convenient at the field station/camping area, break (don't cut to avoid contamination) the core in the following pieces and store them in separate paper bags, each labelled differently to be able to identify the pieces as originating from different parts of the stem.
  - a. **FOR FUTURE RESEARCH:** Bark
  - b. **FOR STABLE ISOTOPES:** Outermost 20 cm of the core
  - c. **FOR DART TOFMS AND NIRS:** Innermost 5 cm of the core

If inconvenient, wait to break the cores until back at the lab and if cores were taken out of the straws for drying, put them back in for transport to the lab.

- **FOR WOOD ANATOMY:** Some 20 cm above or below the height where you took the cores (to avoid damaging the tree too much), take a wood sample of at least 1 cm<sup>3</sup> with punch and mallet, chisel and hammer, a saw (cutting a small wedge) or with an increment borer (Fig. 5h). Store samples in small vials with 50-70 % ethanol (essential for tree species with included phloem) or air dry.

---

\* When bark has deep furrows, it is sometimes also possible to just begin coring between furrows.

† Attention is needed when storing cores for DART analysis. More information can be found in chapter 3.2.

‡ In case you are lucky and have access to a field station that is even equipped with an oven, you can also dry the straws at 65 °C.

- **FOR DNA ANALYSIS & POTENTIAL FUTURE BARK RESEARCH:** At a good distance from breast height (to avoid damaging the tree too much and as DNA anyway does not change within the tree, in contrast to the other wood characteristics), hammer with a **3 cm diameter punch** (Fig. 4c) just 1 cm deeper than the bark (punch the first time rather deep to get to know bark thickness). Take the sample which will contain bark and cambium. If thicker than a few millimetres, separate with a knife the outer layer of bark from the cambium (to speed up desiccation of the cambium<sup>5</sup>) (Fig. 4d). Store both, bark and cambium samples in separate tea bags in a ziplock bag together with a silica gel bag.

**TIP:** In addition to labelling on the outside of vials, small **paper tags** with pencil marks can be put inside the vials. Marker pen labels might accidentally get rubbed off by the ethanol (not all 'permanent' markers are permanent).

**TIP:** To date there is no evidence for the long term benefit of using any **wound treatment after coring** (Grissino-Mayer 2003, Tsen *et al.* 2016). If you are in for an extra research project, you could thus plan more time for the sampling campaign and in one go collect data to study taxa and environmental characteristics that could explain sensitivity to deleterious damage after coring. Tsen *et al.* (2016) have already developed a proforma to quantify and standardise such an assessment.

**TIP:** Use a single, simple lubricant that won't interfere with DART chemical analysis for the increment borer.

### *From already felled trees*

In cases where freshly-felled trees, with still fresh leaves and still lying at the felling site, can be sampled, do not attempt to core. Instead, collect leaves and cambium material from the tree stump (Fig. 4f) [for DNA analysis] and a wood piece of min. 1 cm<sup>3</sup> and ideally 1 x 7 x 11 cm including both sapwood and heartwood from the tree stump [for wood anatomy]. Two other big samples are taken, one sample of 10 cm in radial length, 1 cm thickness and 5 cm wide [for stable isotopes] and one sample of 10 cm<sup>3</sup> of only heartwood [for DART TOFMS, NIRS]. For small trees an entire 2 cm thick disc could be cut. Air-dry samples.

---

<sup>5</sup> To improve the amount and quality of DNA that can afterwards be recovered from the sample.

# 3. Transport & Storage of samples and data

## 3.1 Forest-to-lab sample chain & sample quality

Tips to mitigate the risk for a reduced sample quality due to a long path, with many handling steps of the samples from forest to lab:

- Data from the sampling campaign (notebook data and/or completed sample record templates, GPS coordinates, photos, ...) need to be digitized and well organised as soon as possible and cross-checked with the samples.
- The above should ideally be done by the field team itself (hand writing might be difficult to read)
- GPS coordinates should be checked with online services such as Google Earth.
- Contact information from the field team should be available to answer any question during sample processing in the laboratory.

## 3.2 Sample storage in the field

① Correct sample storage is a critical step to avoid damage or staining caused by bacteria, fungi or insects, which would make the samples useless. Fungal infection of the samples is a considerable problem, especially in tropical countries, so correctly storing the sample in the field is essential during the collection trip. Stains hinder wood anatomical identification and micro-organisms influence the genetic and chemical characteristics of the wood. Also the storage material can influence the chemical characteristics of the wood. Therefore, special care needs to be taken when sampling for DART analysis (see Box 2).

### *Storage advice for the various materials collected*

- The **herbarium specimens** should be checked regularly and the newspapers exchanged with dry ones when damp to prevent the growth of mould\*. In the tropics, when staying longer than two days in the field, herbarium material is best preserved by putting the specimens in a plastic bag wetted† with 50 % alcohol. Back in the lab the herbarium specimens can then be dried in an oven.
- Full length **cores** are stored in paper straws and dried by keeping them 20-30 cm above a small fire (Fig. 5d) or in an oven at 65 °C (if you have access to one). If none of the above is possible they should be taken out of the straws and air dried. If cores are stored in plastic straws or small diameter pvc tubes, they certainly must be taken out immediately when back in the field station/lodging area to leave the cores air drying to prevent them from moulding. Cores can be soaked in sodium hypochlorite before putting in the straws to delay fungi formation. Plastic straws can also be cut open lengthwise to leave the moisture out. Alternatively, cores can already be broken in their pieces at the field station and then stored in tea bags inside ziplock bags with silica gel.
- **Samples for NIRS** should not come into contact with any chemical product (*e.g.* glue at the sticky side of tape).
- Leaves and cambium **samples for genetic analyses** should be stored in ziplock bags or small tubes with silica gel, or in tea bags inside ziplock bags and/or inside airtight plastic containers with a silica gel bag.
- The **wood samples for wood anatomical analysis** and the extra set of flowers and/or fruits (next to the ones attached to the herbarium specimen that is stored between newspapers in a botanical press) are stored in ziplock bags with silica, or in vials with 70 % ethanol, or air dried and stored in paper bags. However, for species with interxylary phloem only storage in 70 % ethanol will prevent phloem cells from collapsing.
- For the same reason **bark** should be preserved in 70 % ethanol.

---

\* A field dryer can be built at low cost that can speed up the drying process if there is enough sunlight (Sinnott 1983).

† Add enough alcohol to prevent the growth of mould but be careful to not add too much alcohol, which could damage the newspaper and the sample.

## General storage instructions

- **To avoid the risk of unidentified bags** lying around, it is good practice to label the bag and to put a paper label written in pencil inside the bag. This will also prevent labels from disappearing due to rain or leaking ethanol.
- All samples stored with silica gel should be checked regularly. Ideally the silica gel is checked in the evening after collection and the latest after 24 h and replaced when it has changed colour with **new, clean silica** (for recycled silica, see Box 1). Check the bags every 24 h for evidence of changing colour in the silica. If it has changed, the silica is replaced. Keep checking bags until no further change in silica colour is observed.
- **If it is impossible to stick to the recommended ratio of 1:10 when using silica gel**, it is better to air dry samples (although not recommended for cambium) as samples will mould inside the ziplock bags.
- All ziplock bags should be kept in a **hermetic** plastic box and/or in a dry area (air conditioning) since ziplock bags are not air-tight.

### BOX 2. STORAGE ESSENTIALS FOR DART ANALYSIS

There is a **potential issue of paper bags or paper straws** for storing wood samples for DART analysis, as the paper can strongly influence the chemical signature of the wood. However, for dense and/or dark wood this is less of a problem. The denser the wood, the less likely it is to absorb the volatile molecules from the paper. The darker the wood, the more likely it is that the inherent richness of small molecules in the wood overwhelms the paper chemical signature.

The general recommendation is therefore to use **paper straws and/or bags of a single defined source**. In addition, a few straws/bags should be kept apart so that the storage material as such can be analysed.

Alternatively, samples can be stored in something that is more **chemically inert** (*e.g.* plastic, aluminium,...). However, special care should be taken to dry samples as fast as possible to prevent mould growth, which will influence the chemical signature without doubt.

Little **research** has been done so far in this respect, some prior investigation is hence recommended. The chemical signal of the storage material might disappear when samples are left to the air for some time before doing the DART analysis. Dissecting the sample so that the sliver used for analysis excludes wood that has been in physical contact with the storage material may work as well.

### 3.3 Sample transport

- Check the phytosanitary rules of the country of import if you are exporting samples out of the country.
- Choose reliable companies to transport the samples and do not forget to provide the research and export permits for transportation.
- If there are samples stored in ethanol and if this would cause a problem for transport, empty the vials for the transport and refill them with ethanol as soon as they arrived at destination.

### 3.4 Long term sample storage

- Deposit the herbarium specimens in a herbarium.
- Store duplicate wood material in a xylarium, with the associated herbarium specimens to assist taxonomic identification (xylarium and herbarium preferably in the same institute).
- At arrival in the laboratory all samples should be further dried if necessary and stored in a cool, dry place (18-22 °C; 45-55 % RH) or in the freezer at -20 °C.
- Special care should be taken on sample labelling on the storage boxes and to the maintenance of documentation and databases.
- If possible, the local partners should keep a duplicate of each sample in case something goes wrong during shipping. In general, it is advisable to keep backup samples in several different laboratories and repositories.

**TIP:** For NIRS, storing wood samples at 20 °C and 65 % RH would be ideal but is not mandatory.

# 4. References

- Bergo, M. C., T. C. Pastore, V. T. Coradin, A. C. Wiedenhoeft and J. W. Braga (2016). "NIRS Identification of *Swietenia Macrophylla* is Robust Across Specimens from 27 Countries." *IAWA Journal* **37**(3): 420-430.
- Blanc-Jolivet, C., Y. Yanbaev, B. Kersten and B. Degen (2018). "A set of SNP markers for timber tracking of *Larix* spp. in Europe and Russia." *Forestry: An International Journal of Forest Research* **91**(5): 614-628.
- BOLFOR/PROMABOSQUE (1999). *Guía para la Instalación y Evaluación de Parcelas Permanentes de Muestreo (PPMs)*. 47 páginas.
- Chaves, C. L., B. Degen, B. Pakull, M. Mader, E. Honorio, P. Ruas, N. Tysklind and A. M. Sebbenn (2018). "Assessing the ability of chloroplast and nuclear DNA gene markers to verify the geographic origin of *Jatoba* (*Hymenaea courbaril* L.) timber." *Journal of Heredity* **109**: 1–10.
- Deklerck, V., K. Finch, P. Gasson, J. V. d. Bulcke, J. V. Acker, H. Beeckman and E. Espinoza (2017). "Comparison of species classification models of mass spectrometry data: Kernel Discriminant Analysis vs Random Forest; A case study of *Afromosia* (*Pericopsis elata* (Harms) Meeuwen)." *Rapid Communications in Mass Spectrometry* **31**(19): 1582-1588.
- Dormontt, E. E., M. Boner, B. Braun, G. Breulmann, B. Degen, E. Espinoza, S. Gardner, P. Guillery, J. C. Hermanson and G. Koch (2015). "Forensic timber identification: It's time to integrate disciplines to combat illegal logging." *Biological Conservation* **191**: 790-798.
- Ekué, M. (2014). *Global Timber Tracking Network STANDARDS AND GUIDELINES. Identification of timber species and geographic origin. Version 1.0.* , [www.globaltimbertrackingnetwork.org](http://www.globaltimbertrackingnetwork.org).
- Emdadi, Z., N. Asim, M. A. Yarmo, M. Ebadi, M. Mohammad and K. Sopian (2017). "Chemically Treated Rice Husk Blends as Green Desiccant Materials for Industrial Application." *Chemical Engineering & Technology* **40**(9): 1619-1629.
- Espinoza, E. O., M. C. Wiemann, J. Barajas-Morales, G. D. Chavarria and P. J. McClure (2015). "Forensic Analysis of Cites-Protected *Dalbergia* Timber from the Americas." *Iawa Journal* **36**(3): 311-325.
- Grissino-Mayer, H. D. (2003). "A manual and tutorial for the proper use of an increment borer." *Tree-Ring Research* **59**(2): 63-79.
- Helmling, S., A. Olbrich, I. Heinz and G. Koch (2018). "Atlas of vessel elements." *IAWA Journal* **39**(3): 249-352.
- Jennings, L., R. Hope and X. van der Burgt (2018). "Making herbarium specimens." *Royal Botanic Gardens, Kew Technical Information Sheet* **15**.



- Jolivet, C. and B. Degen (2012). "Use of DNA fingerprints to control the origin of sapelli timber (*Entandrophragma cylindricum*) at the forest concession level in Cameroon." Forensic Science International: Genetics **6**(4): 487-493.
- Kagawa, A. and T. Fujiwara (2018). "Smart increment borer: a portable device for automated sampling of tree-ring cores." Journal of Wood Science **64**(1): 52-58.
- Koch, G. and U. Schmitt (2015). Control and Requirements for Internationally Traded Wood and Wood Products – EU Timber Regulation: Wood Identification, Thünen Institute of Wood Research.
- Krottenthaler, S., P. Pitsch, G. Helle, G. M. Locosselli, G. Ceccantini, J. Altman, M. Svoboda, J. Dolezal, G. Schleser and D. Anhuf (2015). "A power-driven increment borer for sampling high-density tropical wood." Dendrochronologia **36**: 40-44.
- Lancaster, C. and E. Espinoza (2012). "Analysis of select *Dalbergia* and trade timber using direct analysis in real time and time-of-flight mass spectrometry for CITES enforcement." Rapid Communications in Mass Spectrometry **26**(9): 1147-1156.
- Liesner, R. (accessed 24 April 2018). "Field Techniques Used by Missouri Botanical Garden."
- Lowe, A. J., E. E. Dormontt, M. J. Bowie, B. Degen, S. Gardner, D. Thomas, C. Clarke, A. Rimbawanto, A. Wiedenhoef and Y. Yin (2016). "Opportunities for Improved Transparency in the Timber Trade through Scientific Verification." BioScience **66**(11): 990-998.
- Neo, L., K. Y. Chong, C. Y. Koh, S. Y. Tan, J. W. Loh, R. C. J. Lim, W. W. Seah and H. T. W. Tan (2017). "Short-Term External Effects of Increment Coring on Some Tropical Trees." Journal of Tropical Forest Science **29**(4): 519-529.
- Paredes-Villanueva, K. (in Prep.) Isotopic characterization of *Cedrela* species to verify regional provenance of Bolivian timber.
- Paredes-Villanueva, K., E. Espinoza, J. Ottenburghs, M. G. Sterken, F. Bongers and P. A. Zuidema (2018). "Chemical differentiation of Bolivian *Cedrela* species as a tool to trace illegal timber trade." Forestry: An International Journal of Forest Research **91**(5): 603-613.
- Paredes Villanueva, K. (2018). Tropical timber forensics: A multi-methods approach to tracing Bolivian *Cedrela*. PhD thesis, Wageningen University.
- Pastore, T. C. M., J. W. B. Braga, V. T. R. Coradin, W. L. E. Magalhaes, E. Y. A. Okino, J. A. A. Camargos, G. I. B. de Muniz, O. A. Bressan and F. Davrieux (2011). "Near infrared spectroscopy (NIRS) as a potential tool for monitoring trade of similar woods: Discrimination of true mahogany, cedar, andiroba, and curupixa." Holzforschung **65**(1): 73-80.
- Sinnott, Q. P. (1983). "A Solar Thermo-Convective Plant Drier." Taxon **32**(4): 611-613.
- Snel, F. A., J. W. Braga, D. da Silva, A. C. Wiedenhoef, A. Costa, R. Soares, V. T. Coradin and T. C. Pastore (2018). "Potential field-deployable NIRS identification of seven *Dalbergia* species listed by CITES." Wood Science and Technology **52**(5): 1411-1427.

Tsen, E. W. J., T. Sitzia and B. L. Webber (2016). "To core, or not to core: the impact of coring on tree health and a best-practice framework for collecting dendrochronological information from living trees." Biological Reviews **91**(4): 899-924.

UNODC (2016). Best Practice Guide for Forensic Timber Identification. New York, United Nations.

Vlam, M., G. A. de Groot, A. Boom, P. Copini, I. Laros, K. Veldhuijzen, D. Zakamdi and P. A. Zuidema (2018). "Developing forensic tools for an African timber: Regional origin is revealed by genetic characteristics, but not by isotopic signature." Biological Conservation **220**: 262-271.

The icons used in the quick guide were designed by [Freepik](https://www.freepik.com) from [www.flaticon.com](https://www.flaticon.com)

# 5. Appendices

## Appendix 1: Illustrations to the sampling guide

### Fig. 1 - Sampling material

**a.** Tags to mark sampled trees (codes are scratched onto the metal and written over in pen), **b.** ziplock bags to store samples with silica gel, **c.** air-tight container to store ziplock sample bags, **d.** colour indicating silica gel beads, **e.** tea bags to fill with silica gel and to store several samples, separately in one ziplock bag, **f.** paper bags to store air-dried wood samples, **g.** plastic pvc tubes with corks to seal ends and plastic straws to store cores, **h.** herbarium press, **i.** herbarium material stored in newspapers inside a plastic bag with alcohol during fieldwork (when drying and pressing can not be done in the field).

### Fig. 2 - Sampling equipment

**a.** GPS unit (*Garmin*), **b.** examples of a clinometer (*Suunto*), altimeter (*Haga*) and hypsometer (*Vertex*) to measure tree height, **c.** punch and hammer to take cambium samples, **d.** increment borer and extractor to take wood samples, **e.** equipment to go with wood borer (napkins, dowel, light oil for cleaning, a protective pouch made of bubble wrap and tape, a cardboard tube for storage). To take herbarium material and leaf samples: **f.** telescopic scissors and normal scissors, **g.** telescopic sharpened hook, **h.** sling shot, **i.** tree climber gear.

### Fig. 3 - Collection and storage of herbarium material

**a-b.** Photographic records of individual trees, **c.** branch sample for herbarium specimen, **d.** field botanist making herbarium specimens at the end of the day (at the research station camp), **e-f.** field botanist packing up completed herbarium specimens and wetting them with alcohol for preservation and protection during the remainder of the field work, **g.** herbarium material can directly be dried and pressed when conditions allow, **h.** final herbarium specimens.

### Fig. 4 - Sampling and field storage of leaf and cambium material

**a-b.** Leaves stored in ziplock bag with silica gel for DNA analysis, **c-d.** sampling cambium material with punch and mallet for DNA extraction, **e.** tree after cambium samples have been taken with cambium samples in ziplock bag with silica gel, **f.** sampling cambium material from a freshly felled tree.

### Fig. 5- Sampling and field storage of wood material

**a.** Collecting a 5 mm diameter wood core with a manual increment borer, **b.** storing core in a straw for transport in the field, **c.** cores stored in plastic straws cut open lengthwise to let the moisture out, **d.** cores stored in paper straws drying above a fire,

**e-f.** removing a stuck increment borer from a tree using a rope: by rotating the borer the tension in the rope increases pulling the borer out, **x** collecting a 20 mm core with a mechanical increment borer, **g.** piece of a core stored in a paper bag, **h.** collecting a 20 mm diameter wood core with a mechanical increment borer, **i.** short 20 mm cores for wood anatomy that will be stored in paper bags for air drying.

Fig. 1

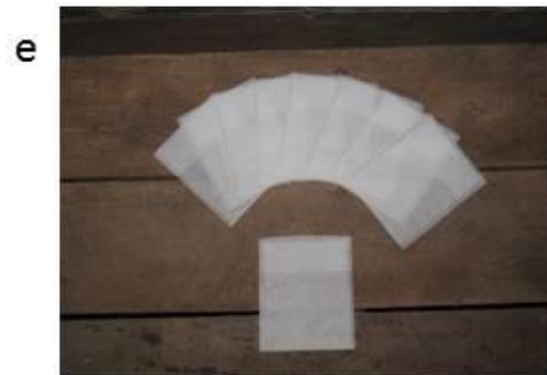
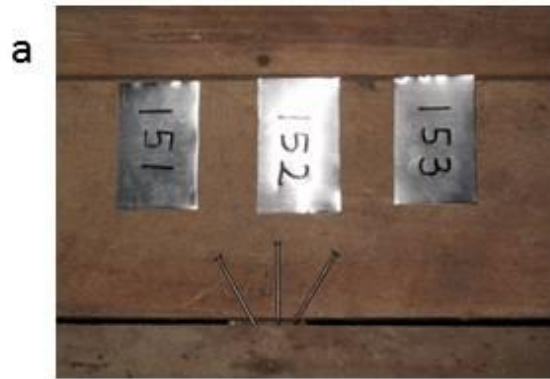


Fig. 2



a



b



c



d



e



f



g



h



i

Fig. 3



Fig. 4





Fig. 5



## Appendix 2: Sampling material & equipment

### Sampling material

- Tags to mark sampled trees
- Permanent marker pens (alcohol and water resistant), pencils, labels, weatherproof notebooks
- Ziplock bags in different sizes
- Plastic containers to store ziplock bags
- Silica gel or rice husk
- Tea filters and/or paper bags in different sizes
- Paper straws, plastic pvc tubes or core holders
- Sodium hypochlorite
- Herbarium press, old newspapers, plastic bag, adhesive tape
- 50-70 % ethanol (to store samples and to clean material), vials

### Sampling equipment

- GPS, cell phone (with GPS Logger app)
- Clinometer and laser meter/measuring tape (or hypsometer)
- Binoculars, camera
- Puncher, hammer
- Increment borer<sup>\*</sup>, napkins, dowel, light oil for cleaning, protective pouch<sup>†</sup>
- Machete, saw, pruning shears
- Telescopic scissors, normal scissors<sup>‡</sup>, slingshot, (tree climbing gear)
- Spare batteries, battery chargers (for GPS, phone, laser meter)

### Personal supplies

- Accommodation
- Food, water
- First aid kit
- Mosquito repellent
- Sunscreen
- Lanterns

---

<sup>\*</sup> Check endnote VII to Table 1 for more information on increment borers.

<sup>†</sup> Can be made of bubble wrap and tape, with a cardboard tube for storage (Fig. 2e).

<sup>‡</sup> To cut branches to the required size for a herbarium specimen.



## Pro forma for collecting timber reference samples for analysis

|  |  |
|--|--|
| <b>Species of timber</b><br>(Scientific name and trade name)   |  |
| <b>Country</b>   |  |
| <b>Region</b>  |  |
| <b>Latitude (or Northing) - MANDATORY</b>  |  |
| <b>Longitude (or Easting) - MANDATORY</b>  |  |
| <b>Alternative co-ordinate system</b><br>(If Lat/Long are not possible, please use this field to give the GPS origin of the sample and give the co-ordinate reference system used) |  |
| <b>Name of collector</b>   |  |
| <b>Date sample collected</b>   |  |
| <b>Approximate diameter of tree</b> (inches or centimetres)  |  |
| <b>Any further comments on location</b><br>(Optional - e.g. on farmland/by a river)  |  |
| <b>Sample number</b>   |  |
| <b>Task Number</b><br>(lab use only)   |  |

### Advice on collecting samples

#### BEWARE - Mould

Whether you are harvesting chips from drilling a tree, cores with an increment bore or a roundel using a chainsaw, please store the timber in a way that allows it to dry (e.g. cotton bags) as timber can go mouldy if left too long.

#### Sample size

The practical approach is the best approach. Drilling or coring a tree often results in a small sample. The best practice is to try to take 2-3 cores per tree at each sampling location. The best type of sample is a cross-section of the tree, but this is not always possible to sample especially in protected areas.

#### How to sample timber:

##### From trees which can be felled

- Please obtain a 'cookie' cross section using a chainsaw from a tree at least 8" wide. Cut the cookie to minimum 0.5" thick.

##### From living trees that can't be felled

- Use an increment bore to obtain a core sample from a tree at least 8" wide, place into a straw (plastic or paper), roll this sample form around the outside and affix with a rubber band. Place sample into a plastic bag.
  - o **OR** use an electric drill with at least 65Nm torque. Drill using an 8" long bore to the core of the tree, try to collect sapwood and heartwood. Collect the chips in a sealable plastic container and affix this form with rubber bands to the container.

#### Ideal sampling site:

Please try to collect samples of timber from 3 trees per location







THÜNEN

With support from



Federal Ministry  
of Food  
and Agriculture

by decision of the  
German Bundestag

[www.globaltimbertrackingnetwork.org](http://www.globaltimbertrackingnetwork.org)

The objective of the Global Timber Tracking Network (GTTN) is to promote the operationalization of innovative tools for wood identification and origin determination, to assist the fight against illegal logging and related trade around the globe. GTTN is an open alliance that cooperates along a joint vision and the network activities are financed through an open multi-donor approach. GTTN phase 2 coordination (2017-2019) is financed by the German Federal Ministry of Food and Agriculture (BMEL).