



WORLD
RESOURCES
INSTITUTE

Using genetic tools to aid in verifying species and origin of commercial timber

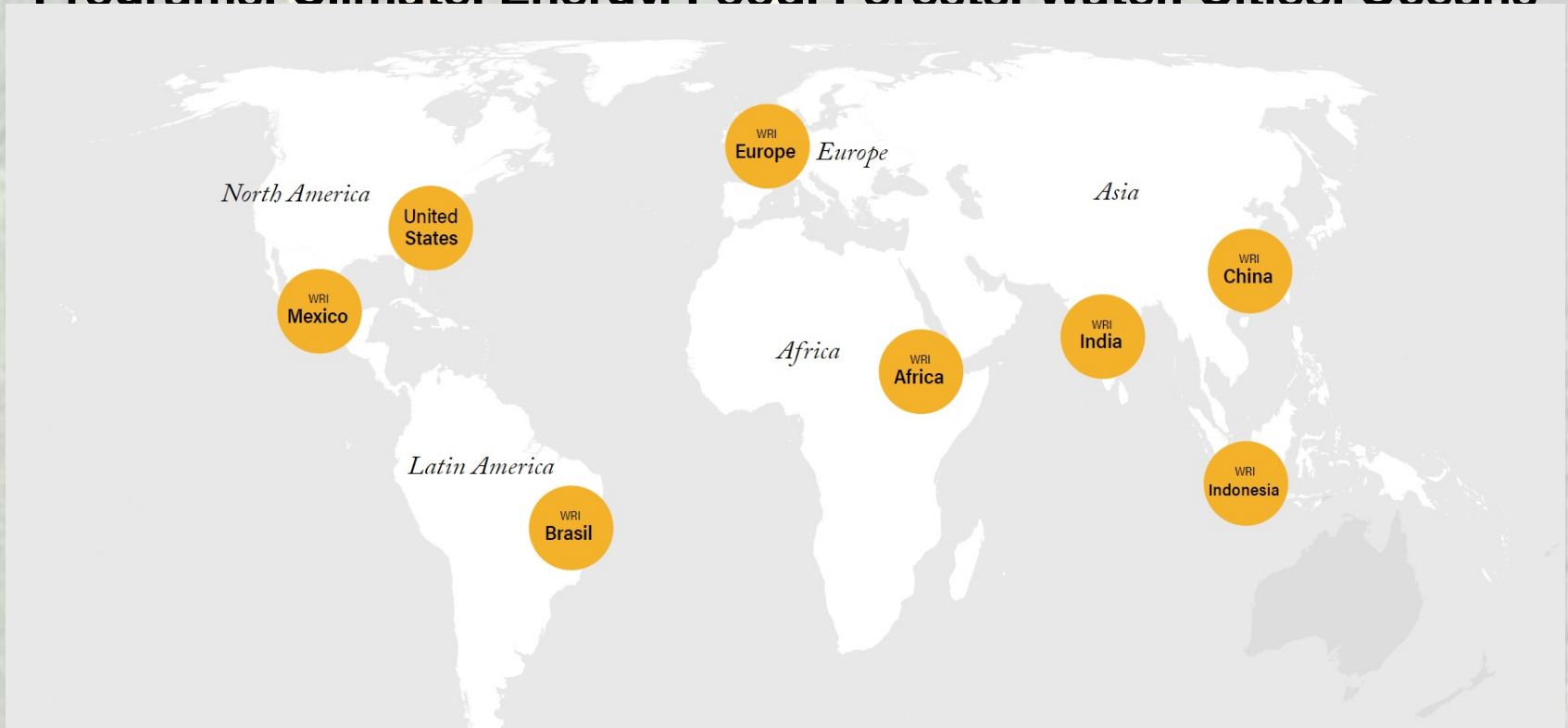
**Meaghan Parker-Forney; Science Officer
Forest Legality Initiative**

***INTRODUCTION TO WOOD IDENTIFICATION TECHNOLOGIES FOR
STRENGTHENING CAPACITIES IN MEXICO SEMINAR
June 17th, 2021***

World Resources Institute (WRI)

A 'Think Tank/Do Tank' research institution focused on the development of applied tools to protect and manage natural resources

Programs: Climate. Energy. Food. Forests. Water. Cities. Oceans



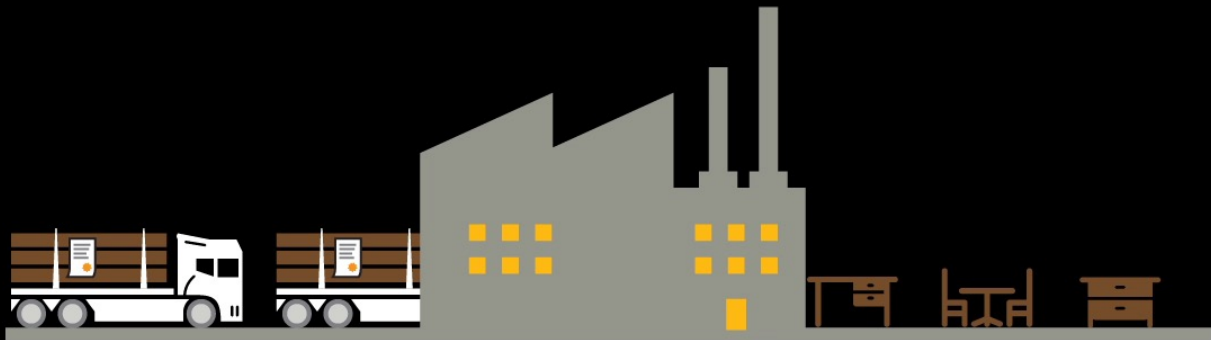
Forest Legality Initiative (FLI)

An initiative within WRI's Forest Program which aims to equip private sector, civil society, government and policy makers with tools and resources for exercising due care and keeping illegally harvested forest products out of the market.

Wood Identification Program

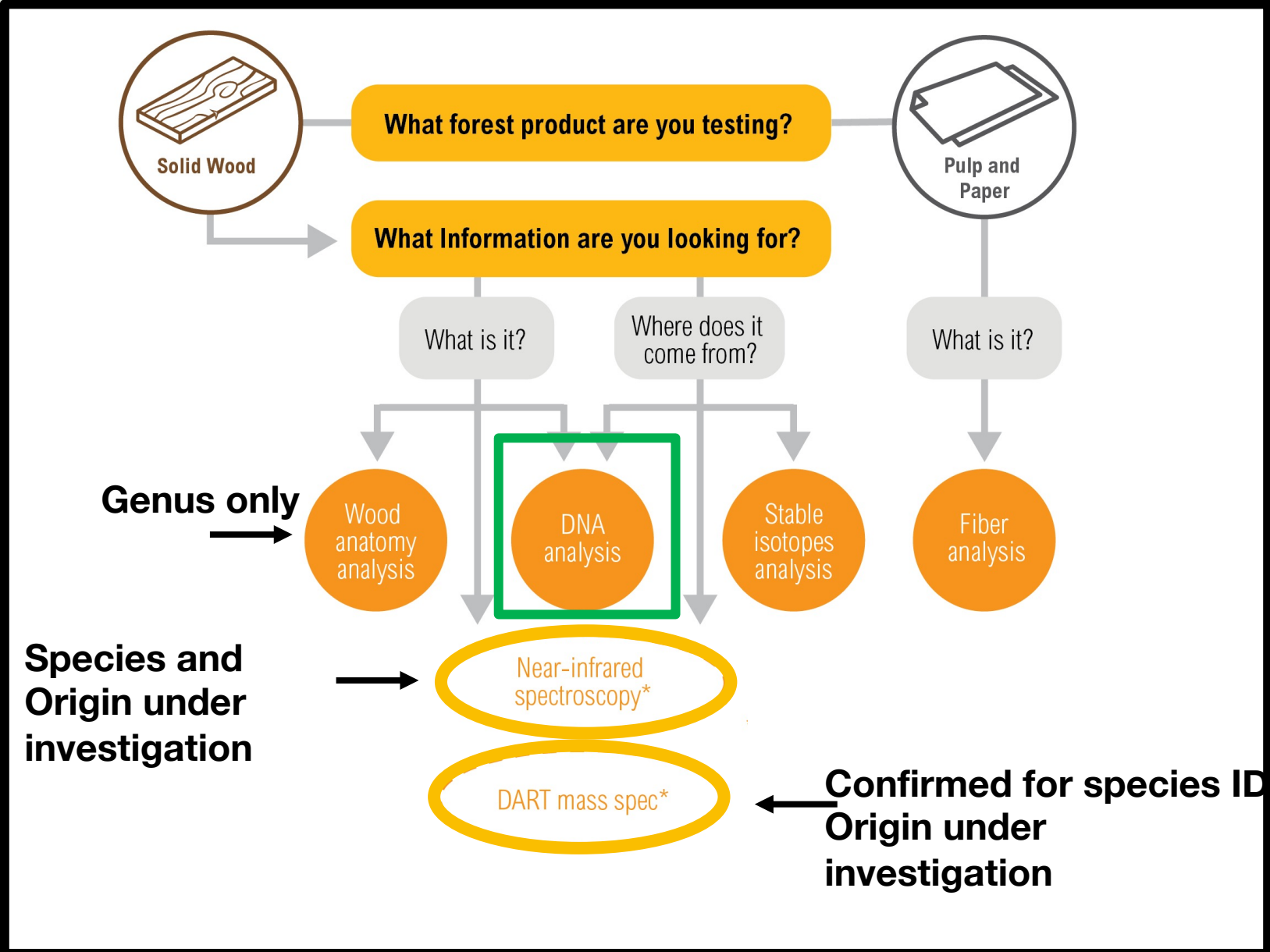
- Works in collaboration with scientists, government/intergovernmental agencies and consortia to develop and execute research projects in wood ID
- Focuses on bringing nascent, open access technologies to scale by collaborating with the public and private sector to deploy pilot projects

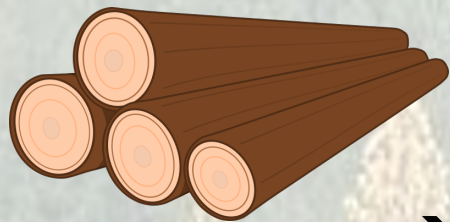
**To expose illegality in the global
timber supply chain....**



**Build tools that validate intrinsic
characteristics of wood products**

Current wood identification methods





Wood



DNA

Species

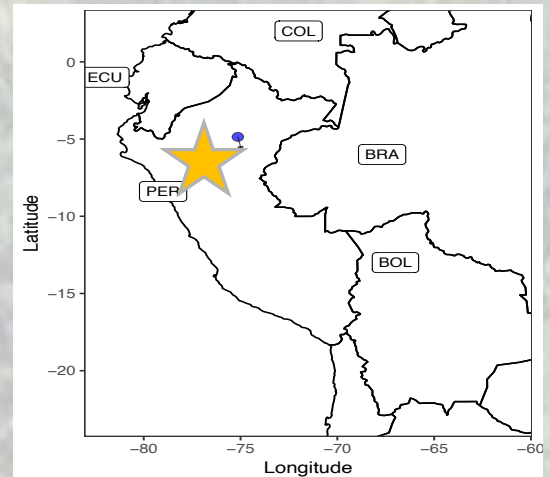


Individual



Leaf

Origin



Validating claims using genetic tools

- Are you looking to identify timber source?
- Are you looking to identify the timber species?
- Are you looking to identify the individual log/stump?

Many different types of genetic tools have been used to answer these questions, but the development of

The development of Single Nucleotide
Polymorphism (SNP) assays have demonstrated
the power to answer all three.

Obstacles to employing genetic tools at scale



Reference databases must first be developed for each method

Enough georeferenced material must be acquired to appropriately address the question(s)

Georeferenced herbarium vouchers must be obtained for verification

Useable DNA from wood products must be obtained

**** SNPs require tiny quantities of DNA (<< 10 ng) and DNA can be degraded***

We can build powerful genetic tools to combat illegal logging

Analogous example in humans (*Harvard's "Science in the News" Blog*)



SEPTEMBER 2, 2018

BLOG

How Direct-to-Consumer Genetic Testing Services Led to the Capture of the Golden State Killer

DNA PROFILING

CRIMINAL IDENTIFICATION



Case Studies

- **bigleaf maple (*Acer macrophyllum*): DNA profiling and source ID**
 - USFS, USFWS, WRI and Adventure Scientists
- **Spanish cedar (*Cedrela odorata*): Species and source ID**
 - USFS and Oregon State University
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 - WRI and Institut Pertanian Bogor (IPB) University

Illegal logging in the United States:

- *~1000 significant timber theft cases/year*
 - *Valued at \$460 Million/year*



Bigleaf maple theft



“figured” maple can be valued at \$400 per board foot

bigleaf maple is a desirable tonewood used in musical instruments

bigleaf maple (*Acer macrophyllum*)

2015 J&L Tonewoods case

“Harold Clause Kupers pleaded guilty to breaking the Lacey Act – the first mill owner ever found to have violated the act by shipping illegal wood across state lines.”

MENU

HighCountryNews



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Busting the tree ring

How a landmark investigation unraveled a Washington timber-poaching gang.

Ben Goldfarb

Image credit: Courtesy Anne Minden

March 20, 2017 | *From the print edition*

The clearing that tree poachers call the Slaughterhouse lies in the northwest corner of Washington’s Gifford Pinchot National Forest, concealed behind the wall of hemlock and cedar that edges Forest Road 25. Ron Malamphy first visited the Slaughterhouse on a damp day near the end of

Acer macrophyllum, DNA Profiling (USFS PNW; Dr. Cronn, USFWS; Forensics Lab)



US Forest Service Law Enforcement REQUEST

- ‘Individualization’ test to determine whether wood blocks at a mill showed a genetic match to 3 illegally harvested bigleaf maple trees from the Olympic National Forest (“ONF”) in Washington, USA.



EVIDENCE

- *Illegal source trees (stumps, leaves)*
- *Milled wood blocks*

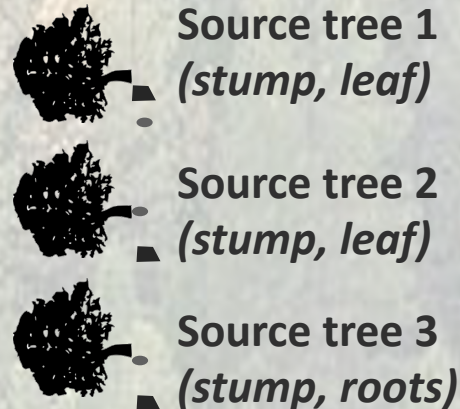
RESPONSE

USFS PNW Research Station geneticists and the US Fish and Wildlife Service Forensic Laboratory:

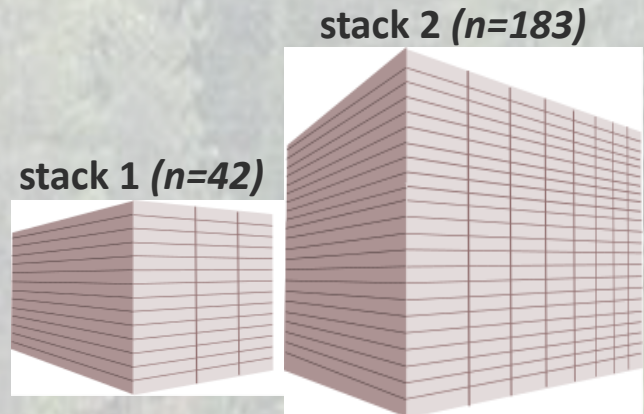
- ***Developed a 'genetic reference database' for bigleaf maple for the ONF***
- ***Extracted DNA from source trees***
- ***Extracted DNA from wood blocks***
- ***Submitted DNAs for SNP genotype analysis to a commercial genomics provider***
- ***Analyzed SNP data and provided reports***



Source trees

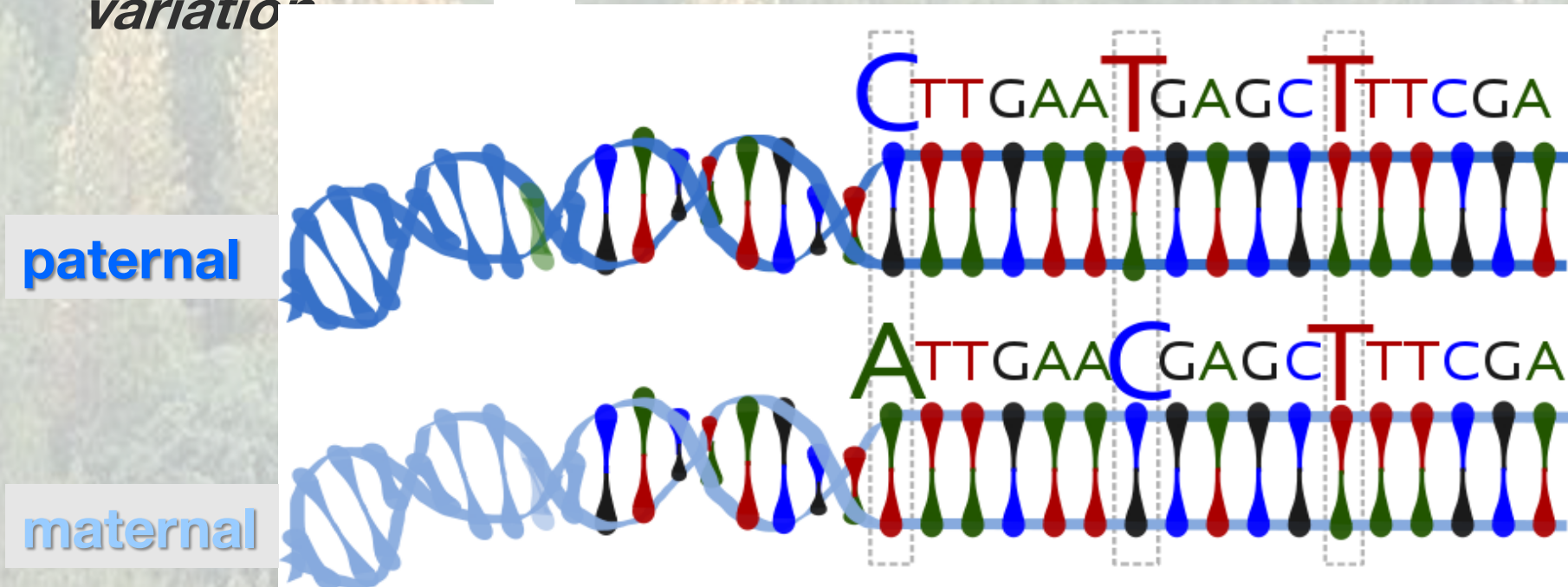


Evidence wood blocks



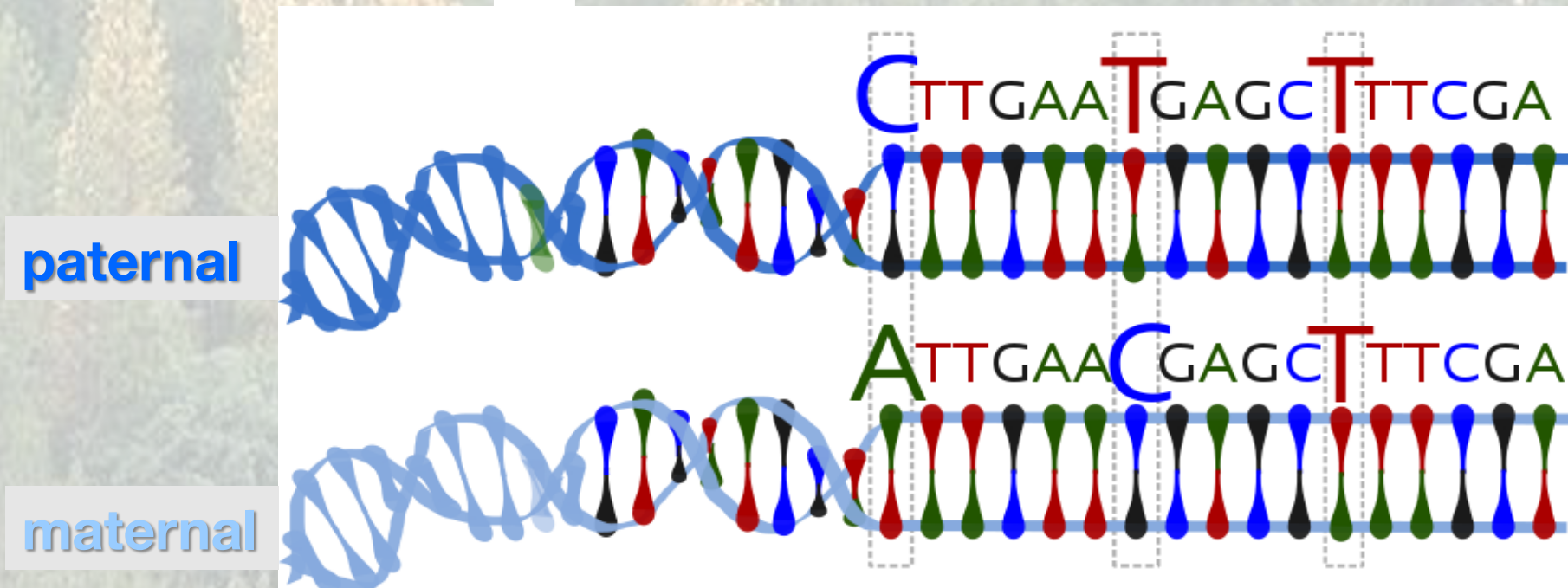
DNA Profiling: The basics

- DNA is double-stranded. Genetic analysis only reads *one strand*.
- Chromosomes occur in pairs (one paternal, one maternal), so DNA analysis includes *pairs of bases*.
- DNA is usually ~99% identical across individuals in tree species, so *effort is required to find DNA variations*.



DNA Profiling: Terminology

- **SNP**: Single Nucleotide Polymorphism, or variation at a specific base location (*e.g.*, C or A).
- **Genotype**: Base sequences at a SNP marker location for an individual (*e.g.*, CA, or TC, or TT).
- **Profile**: Combined genotypes across all SNP markers for an individual (*e.g.*, CA·TC·TT).

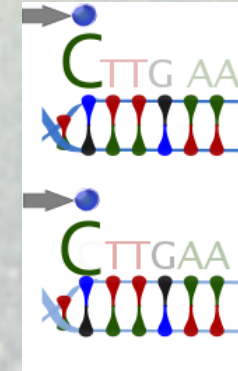
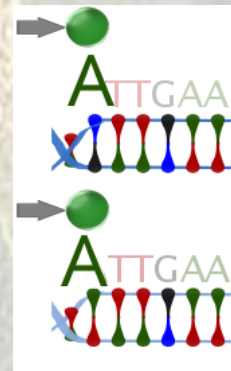
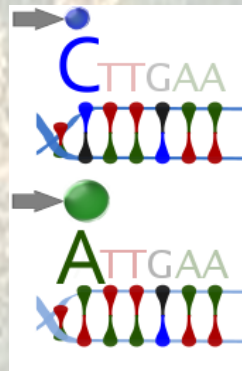


How do we determine profiles?

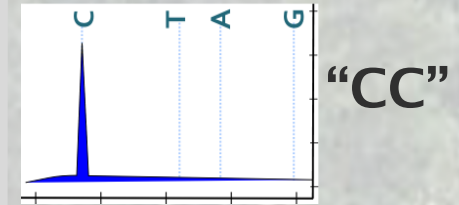
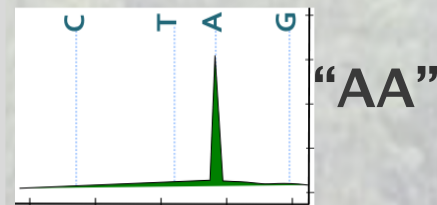
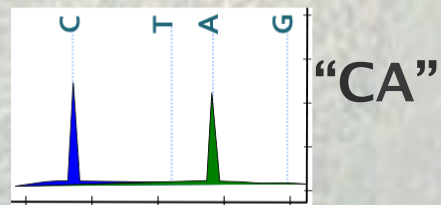
1. Isolate DNA



2. Amplify SNPs



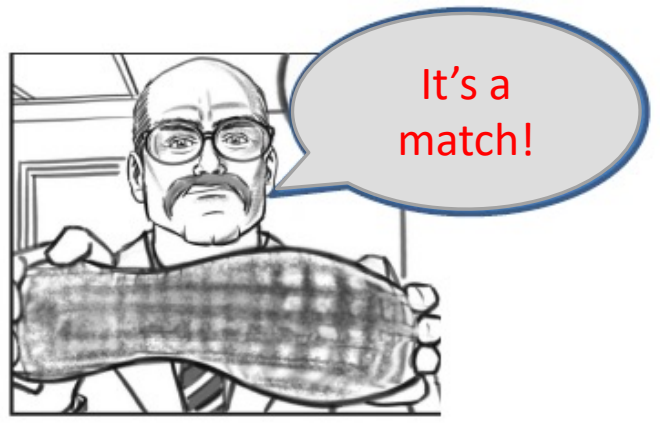
3. Call genotypes



4. Repeat for all SNPs in the panel and analyze

DNA Profiling: What do scientists report?

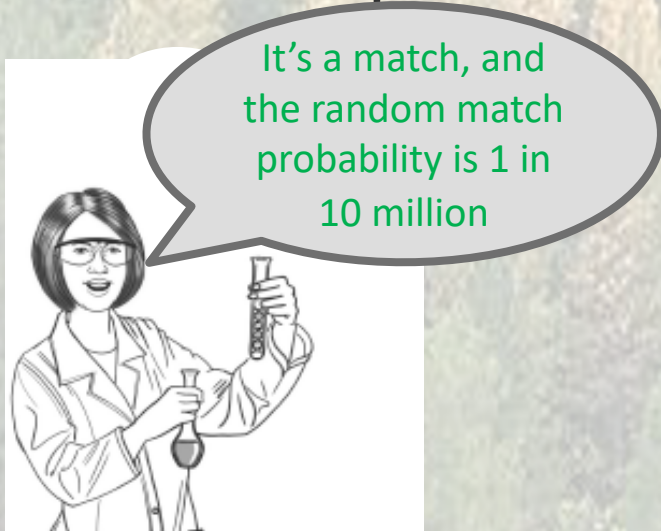
What we **can't** report:



In the analysis, experts **can't** offer opinions about whether items share a common source without making assumptions.

For example: we can't say with 100% certainty how blocks and trees are related because we didn't observe wood cutting or shipping.

What we **can** report:



Experts **can** describe how trees in a forest and wood blocks in a mill are related, based on references to the source (ONF) population.

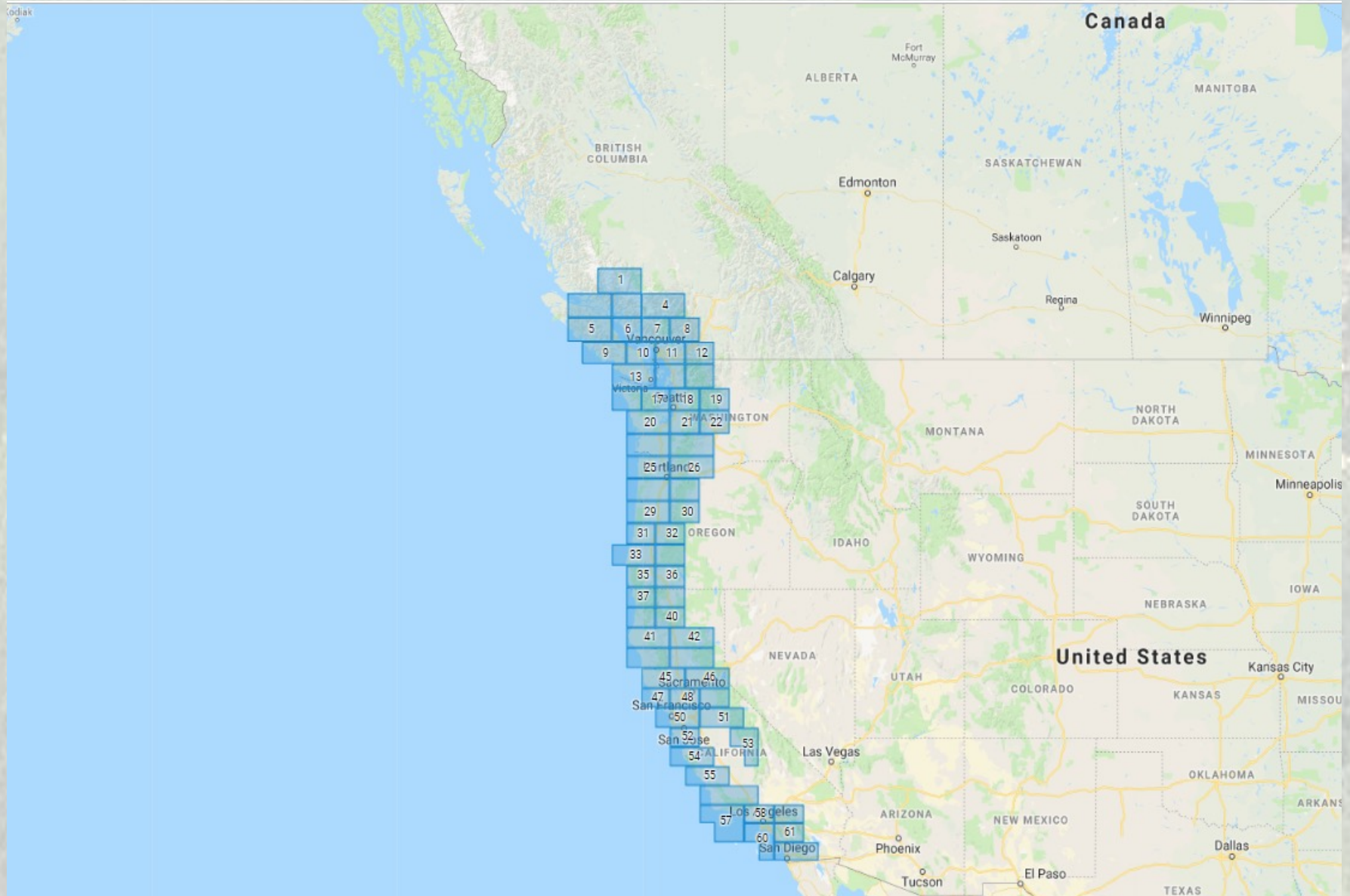
Relationships are based on DNA profiles and reported as probabilities or likelihoods.

Citizen Scientists assist in the development of the bigleaf maple source database

(USFS PNW; Dr. Cronn, WRI; Meaghan Parker-Forney, Adventure Scientists)

- Trained over 120 Volunteers from Southern California to Southern British Columbia
- Collected 1000 samples from 52 regions distributed along 3000 miles of native habitat
- Built SNP database for bigleaf maple
- Developed novel kmer-based analysis method for quick, genomic screening of samples


bigleaf maple collection map




- Developed field collection protocols
- Developed online training programs for collections
- Developed phone apps for field collection data
- Developed analysis platforms for volunteer accessibility

Illegal logging is a global ecological disaster.
Erodes biodiversity · Exacerbates climate change · Bankrolls corruption


Up to 30%* of the international wood harvest is illegal.




Our volunteers collect live tissue samples across the ranges of high-value timber species. First up: bigleaf maple in North America.



Expert geneticists at DNA4 Technologies and New Mexico State University extract, sequence, and catalog DNA from each sample.



This information is used to create a reference library of genetic variation for each tree species across its range.



When DNA is successfully recovered from a suspicious wood sample, it can then be compared to the database to reveal its species and point of origin – illuminating the once invisible differences between legal and illegal timber.

By cracking down on tree poaching, we can protect biodiversity, endangered species, and wild places, ensuring more sustainable forests for everyone.

[* http://wedocs.unep.org/handle/20.500.11822/8030](http://wedocs.unep.org/handle/20.500.11822/8030)

adventurescientists.org/timber

Verizon 19:45

← Wood_Crew Save

1 Scan the leaf sample QR Code that is unique to this collection site. This code should reflect your wood zone number (WZ#), the number of collection sites you've visited, and the sample type (LEAF)

Scan

Verizon 11:07

← Leaf_Crew Save

7 Did you arrive at this site via a publicly accessible route?

Yes

No

8 If no, please describe how you arrived at this location. Are you on private property? Did you need permission to access this site via a private trail/road? If so from whom?

Part I - Wood Crew Training

0% COMPLETE

- INTRODUCTION
- Course Information
- Meet the Timber Team
- PROJECT BACKGROUND
- Project Context
- Project Overview



What to expect from this training course

Thanks in advance for dedicating your time and attention to completing this online training course for the Tracking Timber Project Wood Crew. We've designed this course to prepare you to collect research-grade data over the coming field season. **Please do not forward this e-learning course or the link to the volunteer-only webpage to anyone else —**



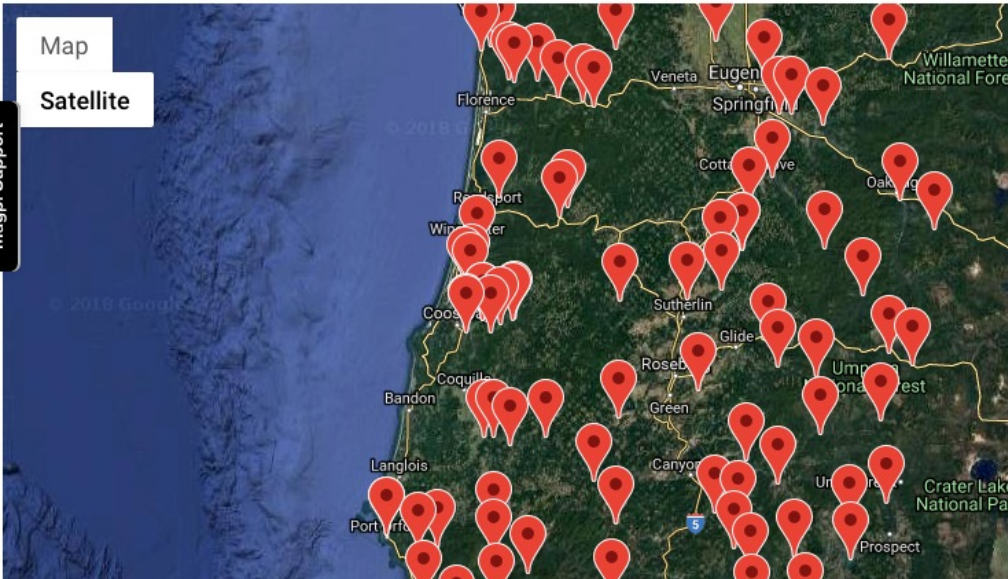
DNA4 Technologies LLC



And Let the Volunteers Loose!

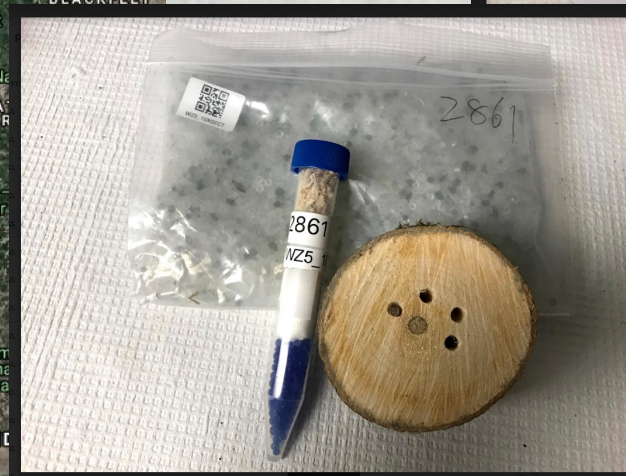
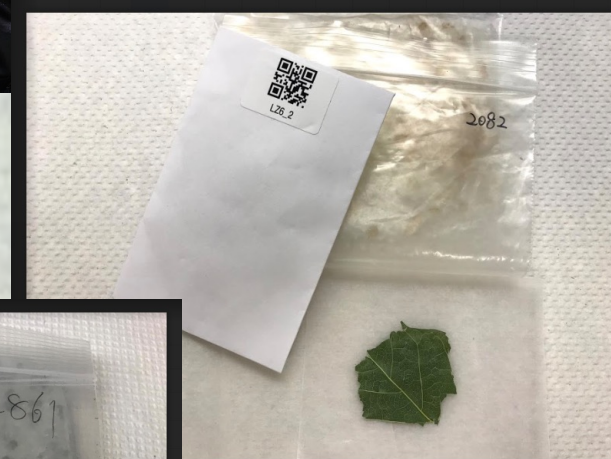
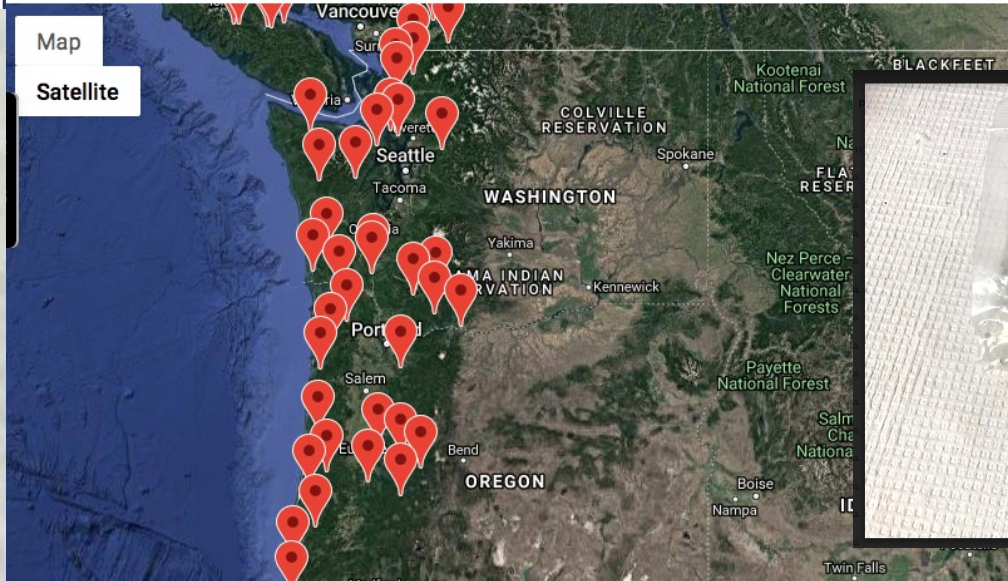
Leaf_Crew

Last edited date from: 2018-05-15 to: 2018-11-18 All Dates... All Use



Wood_Crew

Last edited date from: 2018-06-29 to: 2018-11-14 All Dates... All Users...



IMPACT

- **Enforcement officers in the Pacific Northwest began actively pursuing illegal logging cases of bigleaf maple**
- **U.S. Forest Service began funding the development of chemical and anatomical databases using the big leaf maple samples**
- **U.S. Forest Service has sought funding to develop reference databases for additional high-value timber species (black walnut,**



WHERE ARE WE NOW?

bigleaf



redcedar



coast redwood



yellow cedar



Samples	<input checked="" type="checkbox"/> >1,600	<input checked="" type="checkbox"/> >1,100	<input checked="" type="checkbox"/> ~900	<input checked="" type="checkbox"/> >1,600
Genetic Discovery	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Genetic Analysis	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chemical Analysis	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Databases	Forensics, pop-gen	Forensics, pop-gen	Forensics, pop-gen	<i>N.A.</i> Gene conservation
End-use				

Case Studies

- **bigleaf maple (*Acer macrophyllum*): DNA profiling and source ID**
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- **Spanish cedar (*Cedrela odorata*): Species and Source ID**
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- **Makassar ebony (*Diospyros celebica*): Species and Source ID**
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Cedrela odorata species and source ID

(Oregon State University; Dr. Finch, USFS; Dr. Cronn)

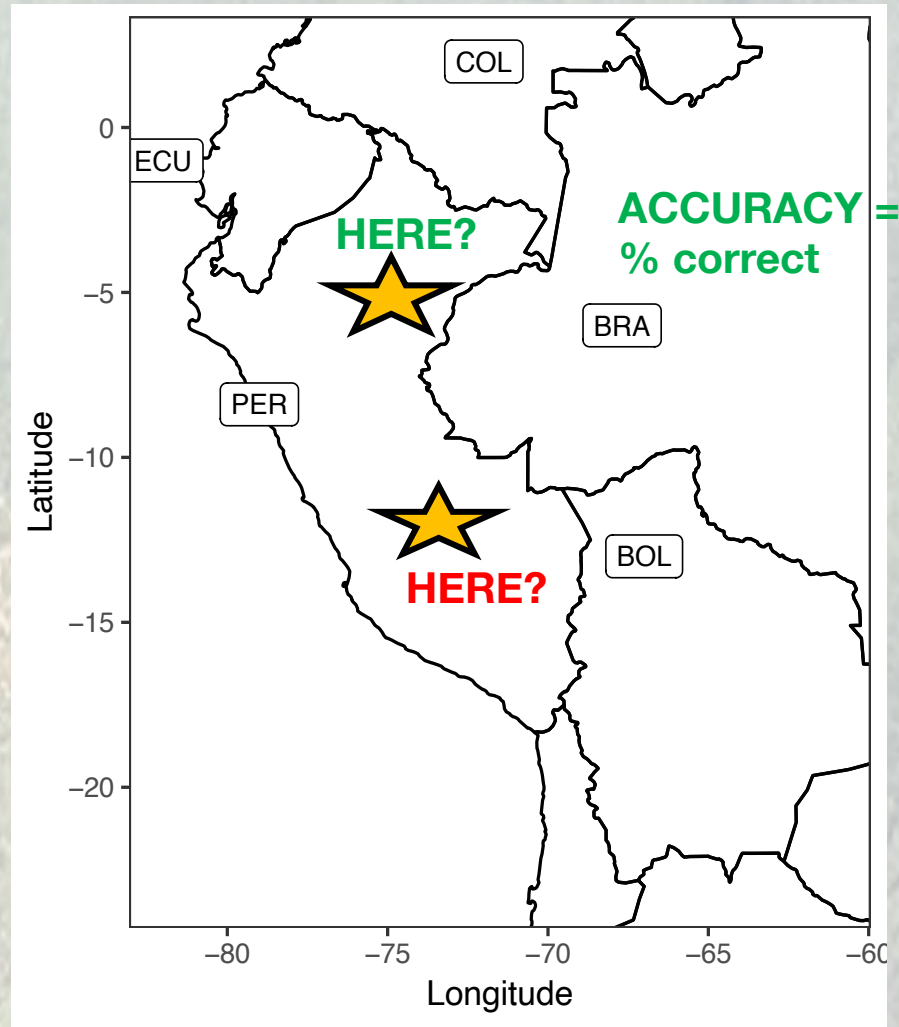
GLOBAL GOAL:

Provide genomic resources for
law enforcement +
conservation
Dissertation Goals:

- Develop genetic tools to ID
Cedrela odorata source
- Understand the
relationship between
Cedrela odorata and allied

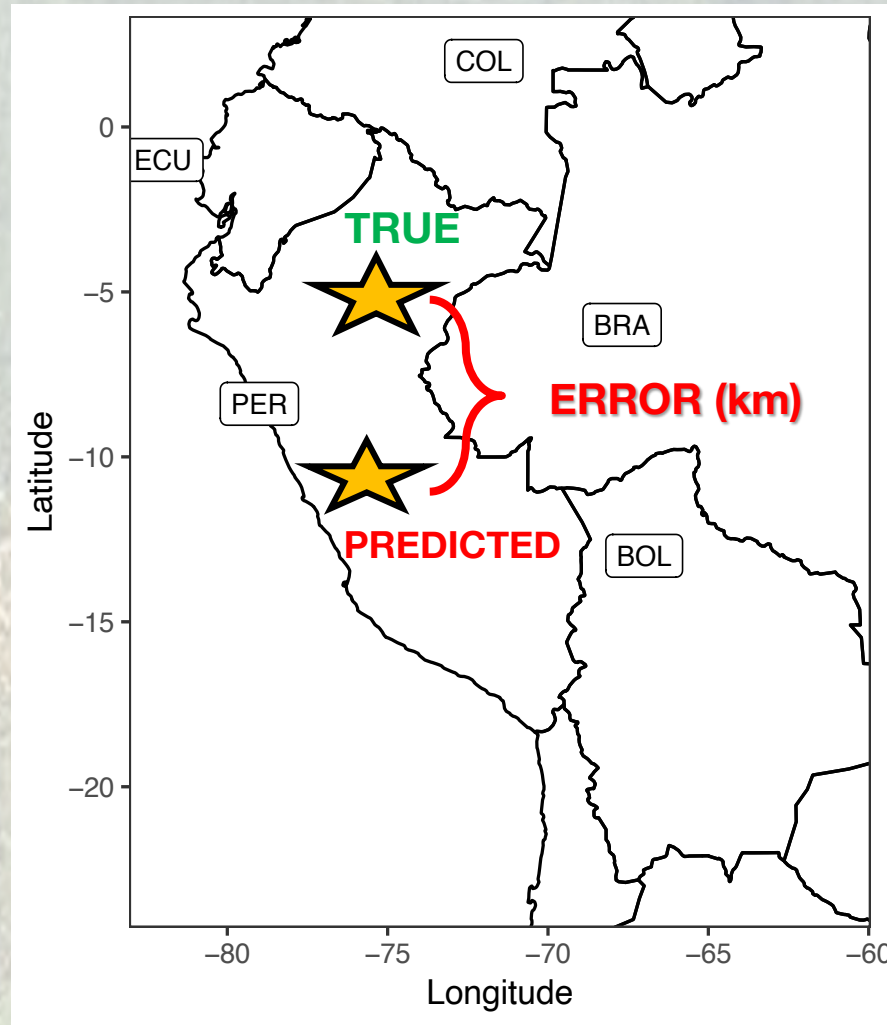


Discrete Classification



Random Forests: *Ask the software is “did the specimen originate here or here?” The % of times it gets the classification correct provides the measurement of accuracy.*

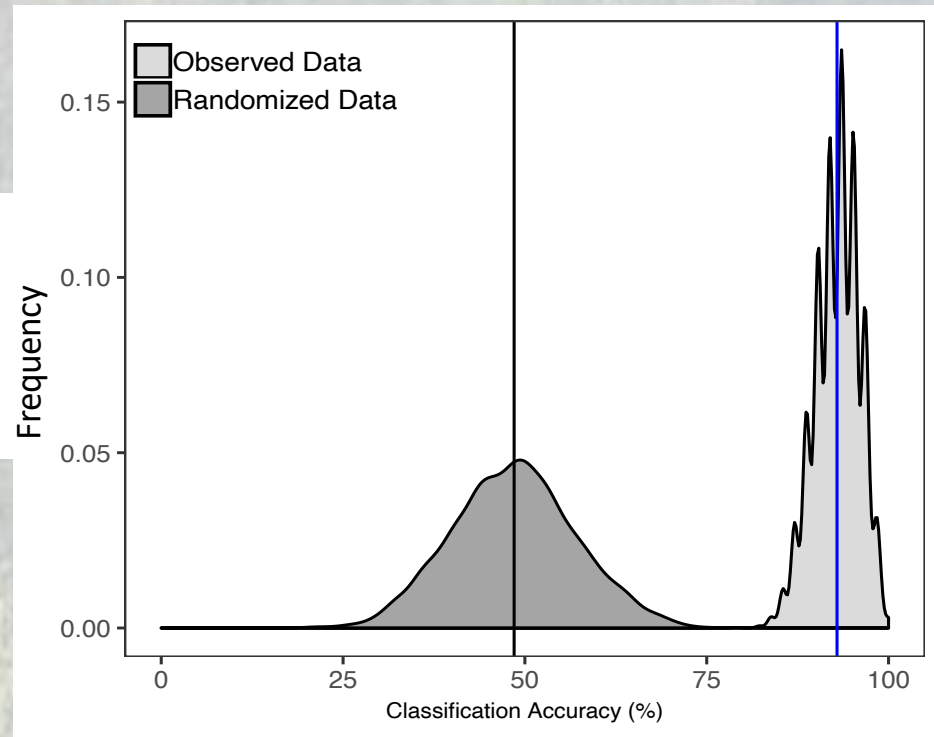
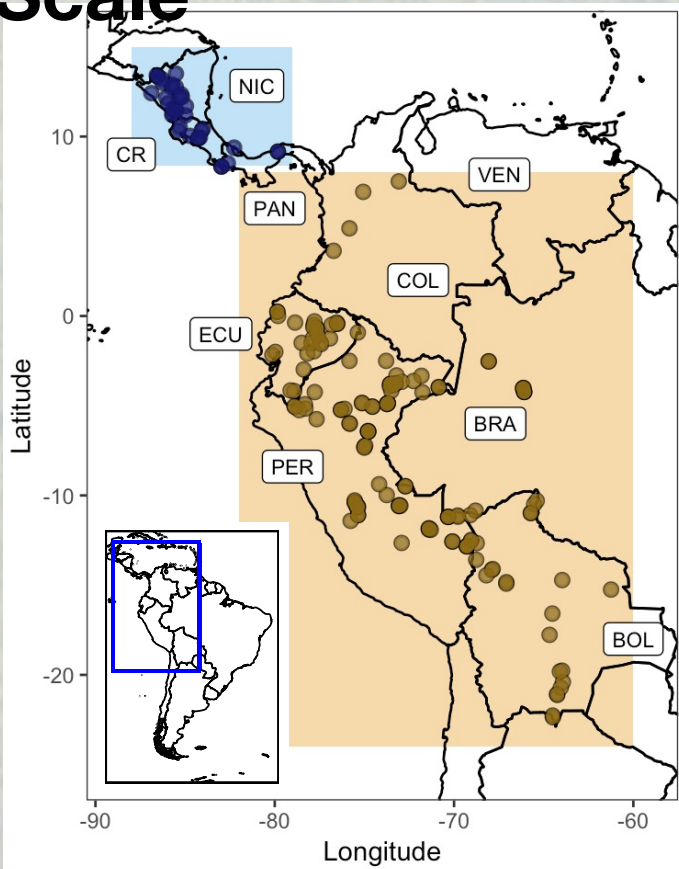
Continuous Prediction



SPASIBA: Ask the software to find the origin. Knowing the true origin, error rates can be determined by distance between true and predicted origins

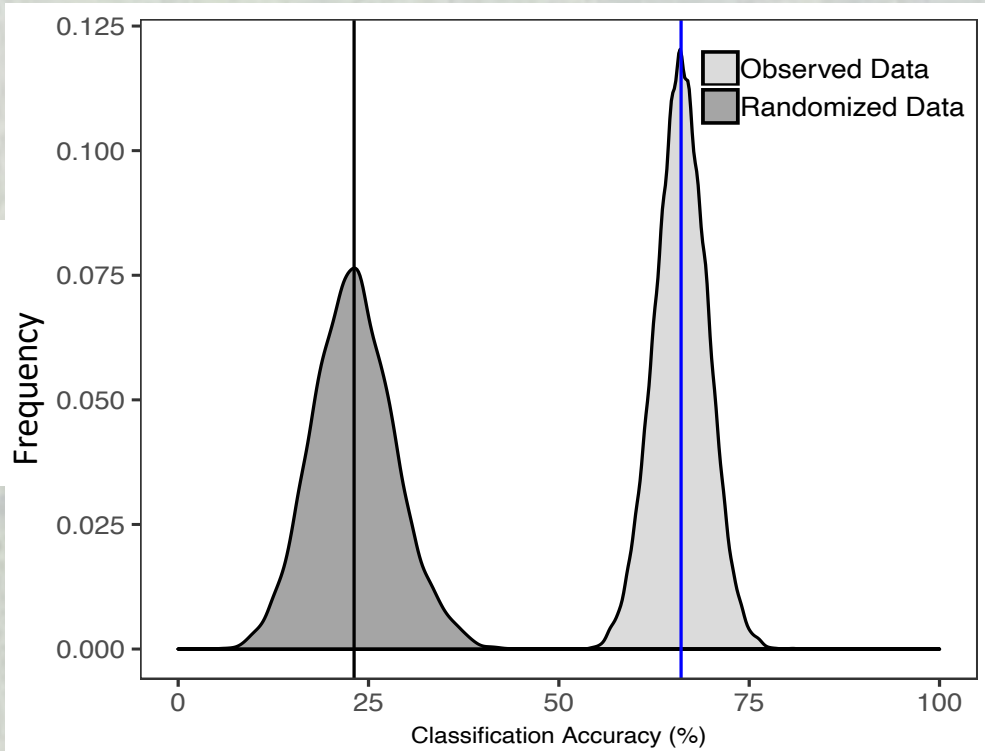
Random Forests (Discrete)

High accuracy at the Continental Scale

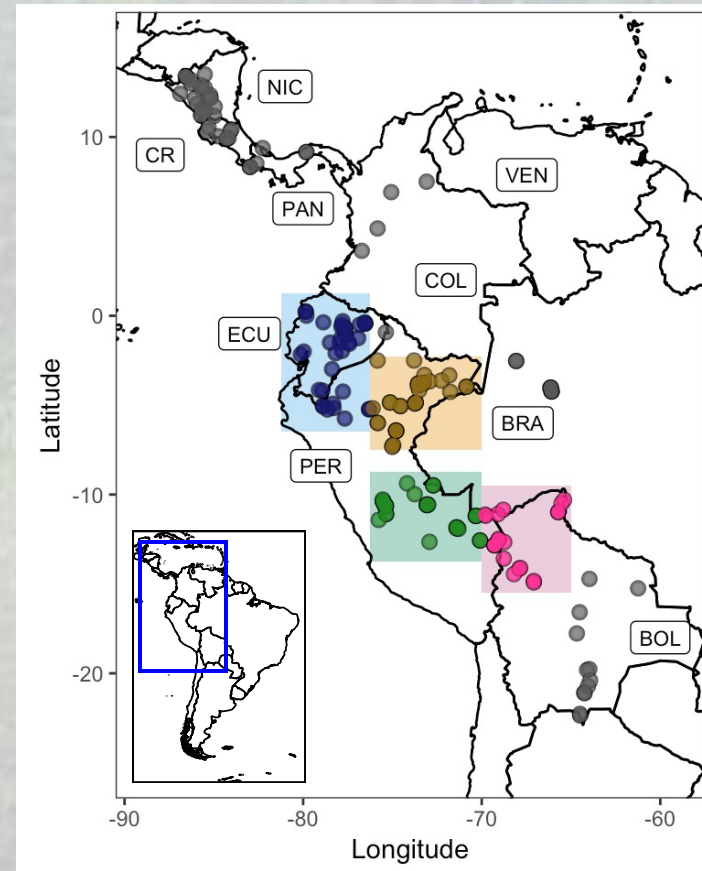


Observed Mean Classification Accuracy	94.2%
Baseline Mean Classification Accuracy	48.9%

Moderate Accuracy at the Small Regional



Observed Mean Classification Accuracy	65.3%
Baseline Mean Classification Accuracy	23.1%

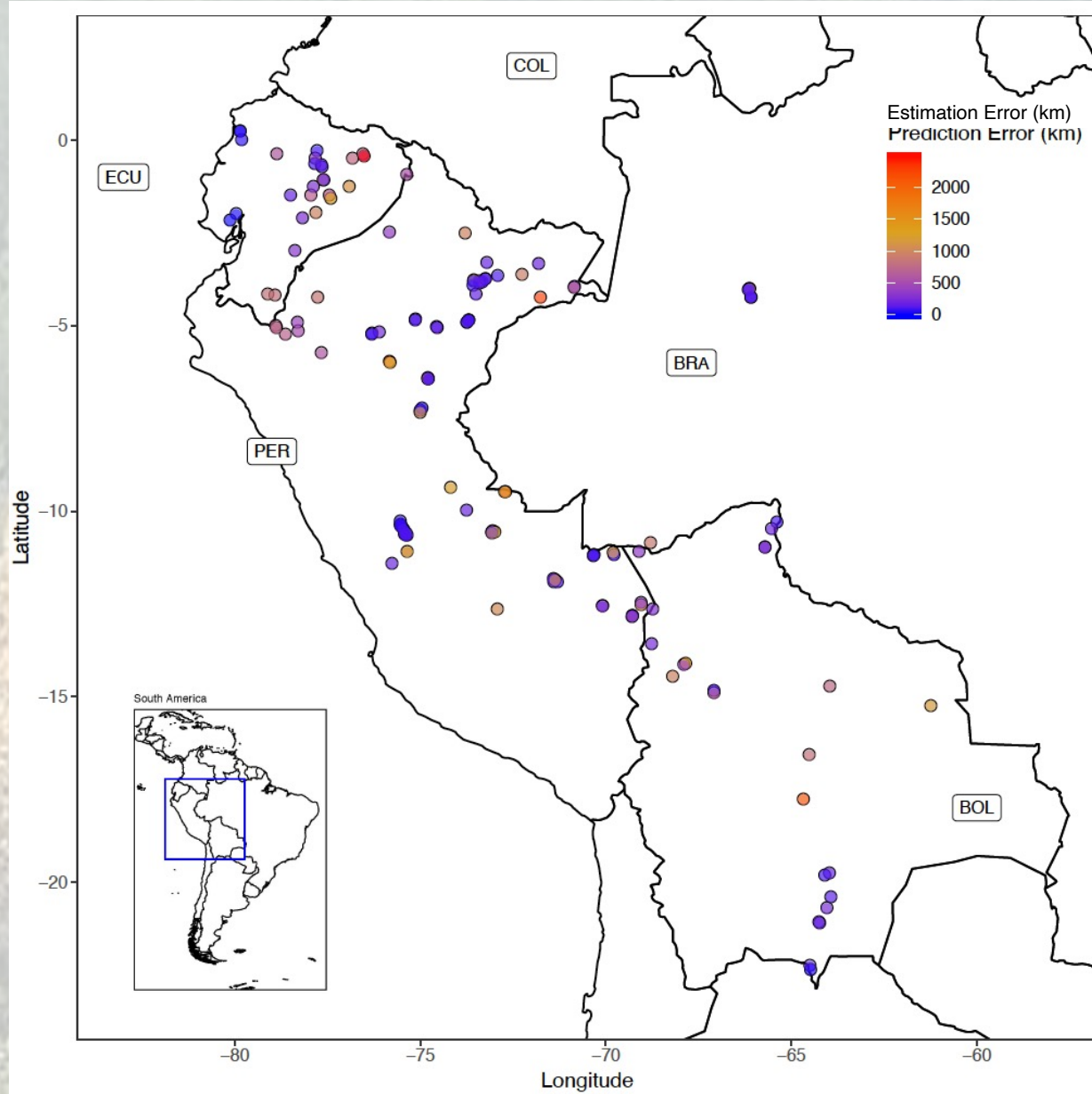


SPASIBA (Continuous)

*Specimens that were assigned near to their true origin are in **blue***

Median estimation error = 188.7km (16.6km – 2472.9km)

****Still useful for enforcement!***



IMPACT

- Can we use DNA to predict *C. odorata* origin? YES!
- High accuracy at the Continental Scale
- Moderate Accuracy at the Small Regional Scale
- Broad range of prediction errors (~17 – 2,500 km) with continuous classification
- Median of 188 km prediction error



Case Studies

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 - WRI and Institut Pertanian Bogor (IPB) University

Diospyros celebica species and source ID

(Institut Pertanian Bogor (IPB) University and WRI)



16 Sites

20 Individual/site

320 Total samples

The second phase will begin in spring 2021

Focus: build genetic database for *Diospyros celebica* plus genetic, anatomical, chemical and isotopic databases for three additional high-value exported timbers



Project Goals (by 2025)

- **Build wood ID capacity in Indonesia using machine vision-based wood anatomy, stable isotope analysis, DART-TOFMS, and genetic analysis.**
- **Assist partner labs in achieving certification with internationally recognized standards by the end of the project**
- **Expand reference databases for three priority species for all wood ID analytical methods**
- **Achieve recognition for wood identification analysis as evidence in enforcement cases**
- **Conduct successful pilots using wood ID methods in enforcement cases to show their reliability.**



Partnerships are KEY!

