

# Science

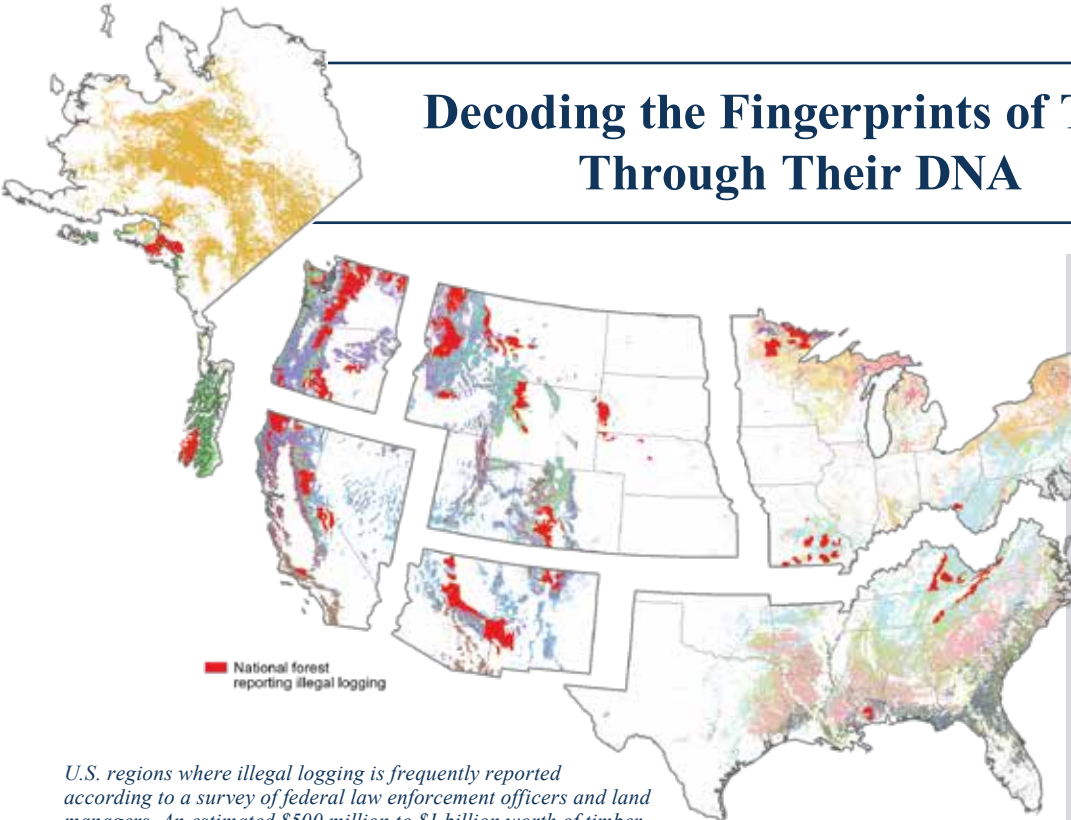
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*"Science affects the way we think together."*  
Lewis Thomas

## Decoding the Fingerprints of Trees Through Their DNA



U.S. regions where illegal logging is frequently reported according to a survey of federal law enforcement officers and land managers. An estimated \$500 million to \$1 billion worth of timber is stolen through illegal logging in the United States each year.

*"You cannot see the wood for the trees."*

—John Heywood, 16<sup>th</sup> century writer

**G**uilty. That was the conclusion of the Maple Fire case of the United States of America versus two defendants. The guilty convictions included theft of public property, conspiracy, depredation of public property, attempting to traffic in unlawfully harvested timber, trafficking in unlawfully harvested timber, and setting timber afire, resulting in the 3,000-acre Maple Fire on the Olympic National Forest that cost \$4 million to contain. Unlike previous illegal logging cases that couldn't be prosecuted due to lack of definitive proof, this case had two pillars of evidence proving that in April through August 2018, the co-conspirators illegally harvested bigleaf maples (*Acer macrophyllum*) from the

Olympic National Forest and sold the wood blocks to an unsuspecting local mill.

First, eyewitness accounts placed the defendants at the scene where the bigleaf maples were illegally harvested. Second, through novel analysis of tree DNA, law enforcement and researchers from the USDA Forest Service matched the stumps to the wood blocks sold to the mill.

The Lacey Act is the primary law used to enforce timber theft in the United States. Passed in 1900 to prohibit poaching or trafficking of fish and wildlife, the act was amended in 2008 to address plant trade and illegal logging. That the United States secured a guilty verdict on illegal logging charges in the Maple Fire case using the Lacey Act is significant.

"The problem we have is proving that the timber came from Forest Service land and the poachers knew it came from Forest Service

### IN SUMMARY

In the United States, the forest products industry loses an estimated \$500 million to \$1 billion annually to illegal logging. Once illegally harvested wood is removed from the forest, it's difficult for USDA Forest Service law enforcement to prove beyond a reasonable doubt that the wood was illegally harvested. The use of DNA is a recent development that can help law enforcement determine if wood is illegally sourced.

Researchers Rich Cronn and Laura Hauck with the Forest Service's Pacific Northwest Research Station developed DNA forensic protocols to evaluate bigleaf maple wood products so that tree DNA is admissible in court. The U.S. Department of Justice used their work to successfully prosecute the illegal harvest of bigleaf maple from the Olympic National Forest. The researchers are expanding forensic tools for other high-value targets of timber theft by collaborating with federal law enforcement, nongovernmental organizations, and citizen scientists to build genomics databases for the species most frequently targeted by timber thieves.

Cronn and Hauck are also exploring other applications for using wood and leaf DNA. For example, this method can determine if trees are clones or genetically different. This information aids in seed collection, enables better tracking of trees grown in breeding trials, and can be used to identify sources of nonlocal trees planted during large-scale reforestation that followed the Hebo and Tillamook Burns in the Oregon Coast Range in the early 20<sup>th</sup> century.

*The 2018 Maple Fire on the Olympic National Forest. The fire was sparked by activities during timber theft and burned 3,300 acres. It cost more than \$4 million to contain.*



land,” explains Seth Wilkinson, an assistant U.S. attorney who prosecuted the case. “What poachers have said in the past is, ‘I got the permission of the landowner to take it off their private property.’ We didn’t have a way to prove that it came from [Forest Service] lands, as opposed to private property.”

Additionally, “Once the log has been sliced up into wood products, such as 2-by-4s, it becomes harder to prove where it came from,” says Phil Huff, a special agent with the Forest Service’s Pacific Northwest (PNW) Region, who assisted with the case.

This is the second federal illegal logging case to use wood DNA as evidence in the United States, but it is the first case to introduce tree DNA in a trial. Significantly, the Maple Fire case establishes precedence that wood DNA can be admitted in future federal timber theft cases.

The Forest Service researchers whose genetic expertise was crucial to the case were also pleased with the outcome. “It was gratifying being able to provide a conclusive answer to Huff’s hypothesis about the source of wood,” says Rich Cronn, a research geneticist with the PNW Research Station.

It’s not widely known that the Forest Service invests in forest tree genomics. “It can be tricky talking about what we do with DNA because it’s not what people think of as forest management,” explains Laura Hauck, a biological science technician with the PNW Research Station. Yet this research is proving invaluable to combat illegal logging both nationally and internationally. It is also finding answers in tree DNA to inform forest management decisions.

## **DNA, Citizen Science, and Sawdust**

Drug busts frequently make the headlines because of the value of the seized product, but timber is an equally high-value commodity that is illegally trafficked around the world. The forest products industry in the United States loses an estimated \$500 million to \$1 billion annually to illegal logging. The International Criminal Police Organization, or INTERPOL, estimates that the equivalent of US\$51 to US\$152 billion of illegally harvested wood is traded worldwide annually.

“Illegal timber in the market depresses global wood prices because illegal traded timber can be sold below market value,” says Beth Lebow, former coordinator of the Forest Service International Program’s Global Illegal Logging and Associated Trade program. “Illegal loggers aren’t following regulation or paying taxes, so it depresses the price of wood and outcompetes legitimate forest products.”

Law enforcement has several tools to determine if timber has been illegally harvested. Wood anatomy can be used to match the grain on cut surfaces of wood. Chemical analysis and anatomy can be used to classify trees to genus and sometimes species. Stable isotopes can determine the approximate geographic origin of wood. Now, DNA profiling can help law enforcement match logs, boards, and even sawdust to their tree of origin. How accurate is this match? Genetic testing confirms that the odds of a random, or incorrect, DNA profile match are less than one in an undecillion (a trillion multiplied by a trillion twice).



*Site of the Maple Fire where one of three bigleaf maples were illegally harvested. USDA Forest Service photos.*

## **PNW Science Findings**

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## Key Findings

- Genetic testing confirms that random matches between DNA profiles in trees is less than 1 in a trillion. With DNA profiling, it is possible to match logs, boards, and sawdust to their tree of origin.
- Through genetic testing, the geographic source of wood can be determined to within 6 to 60 miles (10 to 100 kilometers) from the true source.
- Forest Service researchers and collaborators developed DNA profiling tests to investigate if wood from bigleaf maple (*Acer macrophyllum*) in the Pacific Northwest, Spanish cedar (*Cedrela odorata*) in South America, and Asian white oaks (*Quercus mongolica*) was legally obtained.



An Adventure Scientists® volunteer shows off a maple leaf; such volunteers have made rangewide DNA collections for six species that are targets of illegal logging, including bigleaf maple. Photo courtesy of Christy Carovillano.

Special agent Huff investigated the first U.S. case using DNA to combat illegal logging in 2012, where bigleaf maple was illegally harvested from the Gifford Pinchot National Forest in western Washington. Law enforcement needed to prove that wood at the mill matched the stumps in the forest. A colleague of Huff's heard about DoubleHelix Tracking Technologies, a Singapore-based company able to analyze wood DNA, and reached out to see if they could help with the case.

The company offered to develop a DNA profiling test for bigleaf maple stump and wood samples, but the cost was prohibitive. A call to Forest Service International Programs for assistance led to conversations with World Resources Institute (WRI), a nonprofit group that focuses on developing tools to maintain and restore forests. Through an anonymous donation, WRI funded the project.

When DoubleHelix developed the test and ran the analyses, the samples taken from the stump matched the logs sold to the mill. In this instance, the subjects pled guilty before the case went to trial, but "that was the precedent," Huff says. "It was the first use of DNA in a federal timber theft case, and it was also the first domestic conviction under the Lacey Act for timber. The Lacey Act had been used for wildlife and for timber importing, but never in a domestic interstate trafficking case."

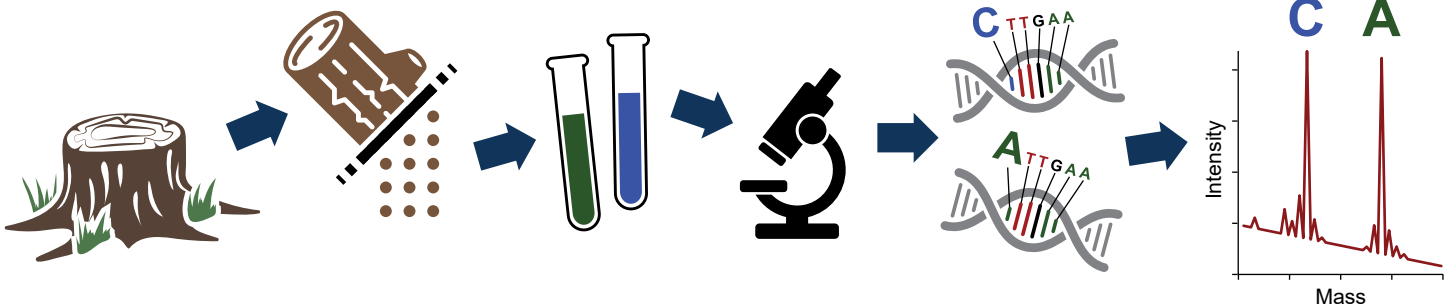
Huff wanted to expand the use of DNA to aid in law enforcement investigations. Meeting Cronn at a conference led them to discuss whether Forest Service research and development could offer genetic testing for Pacific Northwest trees. Cronn welcomed the challenge, and this led Huff and Cronn to reach out to Forest Service law enforcement nationwide to identify other targets of timber theft (e.g., western redcedar and yellow cedar in the west; black walnut and white oak in the east) that would benefit from DNA profiling tests. But Cronn wasn't done with bigleaf maple yet.

### Odds of a random match

- 1 in 0.5 decillion
- 1 in 8 undecillion
- 1 in 29 duodecillion
- 1 in 1.1 undecillion
- 1 in 1.4 decillion
- 1 in 28 duodecillion
- 1 in 2.2 undecillion
- 1 in 2.9 decillion
- 1 in 11 quintillion
- 1 in 11 septillion
- 1 in 12 million
- 1 in 12 thousand
- 1 in 5 thousand

Genetic analysis shows that the DNA profiling test for bigleaf maple has high power in British Columbia, Washington, and Oregon but limited effectiveness in California. Sample locations are shown as dots and are colored by ecoregion; values show the odds of a random match between different samples.

For wood DNA evidence to be admissible in U.S. courts, Cronn and Hauck needed to develop a test that met the standards for forensic trial evidence. These standards are defined by the U.S. Scientific Working Group of DNA Analysis Methods (SWGDM), a group of forensic scientists and forensic DNA laboratories who develop and validate forensic biology methods. "DoubleHelix made a nice initial publication describing their DNA profiling



Wood forensic analysis begins with wood collection at theft sites, log yards, or mills. Once evidence is removed, wood samples are ground under sterile conditions to eliminate the risk of cross contamination, and DNA is released in a mechanical and chemical extraction process. DNA variation is revealed using mass spectroscopy, a process that exploits differences in mass of the four DNA bases (A, C, G, T) to reveal DNA bases present at known positions in the genome. The resulting DNA data can be used for individualization, species identification, or geographic localization.

test, but it was a basic description,” Cronn says. “It didn’t go into detail how it performed as a forensic test,” which is a key requirement of SWGDAM standards.

Early in the process, Hauck found that obtaining DNA from wood posed significant challenges. “It’s easy to get DNA from a leaf, but it was prohibitively difficult to get DNA out of wood,” she explains. “The first step was determining if I could get reliable DNA out of a chunk of wood, not knowing if it was taken from the outer cambium layer, which is the living portion of the wood, or from the middle of the tree, which is dead.”

“Molecular biology meets woodshop class,” is how Hauck described the work. She experimented with sanding and grinding to open cells containing minute amounts of DNA. When case samples were provided as shavings, these samples had to be pressed into pellets so they could then be ground into sawdust to release that DNA from the rigid wood cell structure and be extracted. Once enough DNA was obtained from the samples, the researchers analyzed them using the polymerase chain reaction (PCR) method.

“The PCR method we use targets regions [of the genome] that we know are variable,” Cronn explains. “This process allows us to determine the DNA sequence of the specific locations that we know discriminate individual trees.”

In addition to bigleaf maple, Hauck also developed extraction methods for more challenging woods such as black walnut and western redcedar. “The importance of Laura’s work

## LAND MANAGEMENT IMPLICATIONS

- Timber theft investigations can now include DNA forensic information to verify relationships between wood products and their claimed source.
- The increased use of DNA-based tools can deter timber theft, increase funds to timber-dependent communities through legal sales, and reduce demands on land management law enforcement staff.
- The ability to identify natural clones versus genetically distinct trees is useful for managing seed orchards and breeding populations.
- Methods for geographic prediction of trees can be used by forest managers to verify the source of nonlocal restoration materials. “Off-site” tree sources, such as those used after the Mount Hebo and Tillamook Burns in Oregon in the early 20<sup>th</sup> century, can be confidently identified and managed.

cannot be overstated,” Cronn says. “She really worked out methods to isolate the trace vestiges of DNA that are locked inside dead cells.”

By analyzing the DNA, law enforcement can now ask additional questions about wood evidence, such as “Where did the illegally harvested wood originate?” Cronn has worked with international teams to identify genetic markers that reveal the continental origin of white oak wood (white oaks are native to Asia, Europe, and North America), and the country of origin for South American Spanish cedar, a high-value mahogany relative. Using novel machine-learning methods, Cronn and his collaborators can now use DNA to predict the geographic origin of wood to within 6 to 60 miles (10 to 100 km).

To obtain accurate geographic predictions with DNA, rangewide databases are essential because gaps lead to unexpected errors. Meaghan Parker, the science officer for WRI’s

Forest Legality Team, wanted to expand their work and develop a rangewide genetic database for bigleaf maple that could also be used for geographic prediction. “We know bigleaf maple are being illegally harvested,” she explains. “If we can determine the provenance of the samples being confiscated, then we have a really good shot at getting traction on the enforcement side.”

Parker worked with Adventure Scientists®, a nonprofit who recruits citizen scientists to collect data for researchers, to obtain samples for Cronn and Hauck to analyze. “Within three months, we had volunteers collecting from southern California to southern British Columbia,” Parker says. “They collected a thousand samples along the entire range.” The resulting genetic database can now be used to identify individual trees and predict the origin of bigleaf maple, making an on-the-shelf forensic tool that benefits law enforcement in the United States and Canada.

*Examples of figured wood and a guitar made using wood with flame grain. Trees that make figured wood are targets for timber poaching due to their unusual grain, rarity, and value (up to 100 times more valuable than unfigured wood). Wood photos by Rich Cronn; guitar photo courtesy of PRS Guitars.*

**Quilt**



**Flame**



**Spalted**





Based upon successes with bigleaf maple, Forest Service International Programs has funded new genetic databases for other domestic species, says Beth Lebow. “International Programs thought that this work was important because this wood does enter international markets,” she explains. “It’s obviously a priority with the U.S. government not to have timber theft from federal lands.”

As DNA tools advance and the current chemical and stable isotope tests are refined, the future of tracking wood around the world will be through the wood itself. “Traceability has historically been paper based, and it’s easy to fraudulently doctor paper-based documentation,” Parker explains. “This identification is on an intrinsic level, whether you’re looking at chemical, genetic, or anatomic, isn’t fungible.”

## Information for Future Forests

Decoding tree DNA has uses that go far beyond law enforcement. Cronn and Hauck are applying tree genetics and genomics to answer diverse forest management questions. They are building dense genetic databases that span species ranges and capture genetic diversity across the landscape. When genetic variation is paired with climatic data, such as temperature, aridity, or precipitation, it’s possible to develop prediction models for how populations will respond to climate change. This knowledge will help managers who select seedlings for planting and inform breeding programs that are developing the next generation of climate-resilient trees.

Tree DNA also helps the Forest Service manage tree improvement programs such as the four-decade effort focused on Port Orford cedar (*Chamaecyparis lawsoniana*). Cronn’s lab has developed genetic markers that managers use for quality control to determine if mistakes were made in propagation or labeling trees. He says that’s because after 40 years, “there’s a lot of potential for mislabeling and missing tags.”

The researchers are also applying genetic-geographic prediction to look retrospectively at reforestation in the Oregon Coast Range after forest fires in the early to mid-1900s. Following the 1910 fire on Mount Hebo and Tillamook Burns that spanned 1933 to 1951, land managers planted Douglas-fir (*Pseudotsuga menziesii*) seedlings from any source they could find. Some came from distant seed zones, creating unintentional

assisted-migration studies decades before the Forest Service formally initiated such research in response to climate change.

“There are many planted forests like the Hebo that used off-site trees by accident,” Cronn says. “Now we have a century of growth information gathered on those trees. If we can determine where those trees came from, we will have the necessary information to show how these trees perform in new environments.”

“Leveraging this technology across multiple fields is exciting,” says Hauck. And with the growing opportunities and demands for more genetic information, Cronn adds that continuing to invest in this research is as important as ever.

*“Holy Mother Earth,  
the trees and all nature  
are witnesses of your  
thoughts and deeds.”*

—Winnebago Tribe proverb

## For Further Reading

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*Oregon, Columbia River Gorge National Scenic Area, Latourell Falls and bigleaf maple trees.  
Photo by Danita Delimont.*

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## Writer’s Profile

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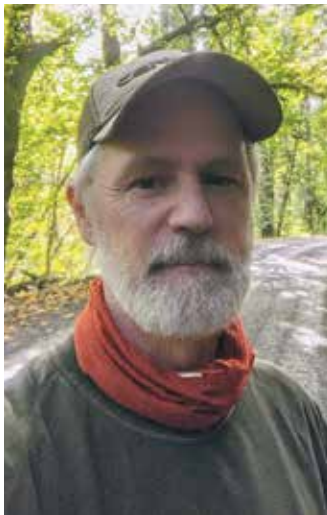


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## ***Scientist Profiles***



RICH CRONN is a research geneticist with the USDA Forest Service Pacific Northwest Research Station. His current research focus includes advancing genetic marker technologies for domestic and international timber to improve legality and to help prosecute timber theft and adopting genomics technologies for applications in forest management, such as environmental DNA. He has a Ph.D. from Iowa State University in plant genetics.

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