

# **BEGINNER'S GUIDE TO DNA TECHNOLOGY AND ITS APPLICATION IN TIMBER TRACKING**

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## **Summary:**

This document has been prepared to introduce the reader to the various DNA technologies that are relevant to Certisource Timber Tracking. The document goes on to perform a comparative analysis of these technologies.

## **Introduction:**

Deoxyribonucleic acid (DNA) is a chemical that contains the genetic instructions used in the development and functioning of all known living organisms. The main role of the DNA molecules is the long-term storage of information and is often compared to a set of blue prints, since it contains the instructions needed to construct other components of cells.

DNA technology is used extensively in the following fields:

### **Genetic Engineering**

This involves the creation of DNA sequences assembled from other sequences. These sequences can be transformed into genetically modified organisms that can be used to produce vital proteins. Such proteins are used extensively in medical, agricultural and pharmaceutical research.

### **Bioinformatics**

This field involves the manipulation, searching and data mining of DNA sequence data. The techniques are used to study diversity amongst organisms; examples such as the Human Genome Project extensively use data mining concepts. By using Bioinformatics, scientists are able to predict the presence of particular gene sequences even before they are isolated experimentally.

### **Nanotechnology**

DNA nanotechnology uses the unique molecular recognition properties of DNA and other nucleic acids to create a self-assembling structure. Also known as the “DNA origami” method, it is used to create DNA machines, which are deployed extensively in biochemical research.

### **Forensics, History and Anthropology**

DNA fingerprinting, DNA testing, DNA typing and DNA profiling are techniques used to distinguish between individuals of the same species using only samples of their DNA. The technique exploits highly variable repeating sequences called micro- or mini-satellites, which are used extensively in criminal forensics and paternity tests.

DNA undergoes mutations over time, which is then passed on to future generations. It therefore contains historical information of the organism and by comparing DNA sequences; geneticists can infer the evolutionary history of the organism.

The techniques of comparing DNA are discussed in the next section.

## **Genetic Fingerprinting Technologies**

All living things contain programmed genetic material called DNA. DNA is made up of a chemical that contains one of the four bases; they are Adenosine, Thymine, Cytosine and Guanine. It is the sequence of these four bases along the backbone that encodes information. This information is read using the genetic code (a set of rules which defines the sequence of the bases). Genetic fingerprinting techniques use a particular element of DNA called molecular markers. Molecular markers don't have any biological effect; instead they are identifiable DNA sequences found at specific locations of the genome, which are transmitted from one generation to the next.

Only a small fraction of the DNA sequence is actually made up of genes (strands that contain code for proteins); a major portion of DNA contains non-coding sequences. The genetic material is organised into sets of chromosomes and the entire set is called the genome. In a plant cell, there are three organelles (organs within a cell) that produce their own DNA, the Nucleus (Brain) referred to as nuclear DNA, the Mitochondria (Energy producer) and the Chloroplast (the food producer). The Mitochondrial and Chloroplast DNA are together referred to as extra-chromosomal DNA.

Different types of molecular marker systems exist: RFLPs, RAPDs, AFLPs, Microsatellites and SNPs. The usefulness of each type varies according to the amount of time and effort required to detect each type of marker, and the extent of genetic variation found at each marker in a population. Each has its own advantages and disadvantages.

### **RFLPs (Restricted Fragment Length Polymorphisms)**

These are markers detected by treating DNA with enzymes (Restriction Enzymes) that cut DNA at specific sequences. RFLPs were the first molecular markers that were widely used, however simpler marker systems have since been developed.

### **RAPDs (Random Amplified Polymorphic DNA)**

These markers were first described in 1990 and detected using a technique called Polymerase Chain Reaction (PCR). This procedure enables the production of multiple copies of specific DNA sequences (amplification). DNA analysis is much quicker than RFLP but the results are highly sensitive to laboratory conditions.

### **AFLPs (Amplified Fragment Length Polymorphism)**

AFLP is another PCR based method of generating molecular markers. In this technique DNA is treated with restriction enzymes and amplified with PCR. This allows selective amplification of certain sequences, which gives rise to a large number of useful markers which can be located on the genome relatively quickly and reliably.

### **Microsatellites**

These are simple DNA sequences repeated a variable number of times in tandem. They are easy to detect with PCR and a typical Microsatellite marker has more variants than those from other marker systems. On the down-side, initial identification of micro satellite markers is time-consuming and expensive.

### **SNPs (Single nucleotide polymorphisms)**

These DNA sequences have become an increasingly important class of molecular marker. The potential number of SNP markers is very high and procedures have been developed for automatically identifying SNP loci (a location point in the DNA) at a very low cost per sample.

## Usage of Molecular Markers in Timber Tracking

Table 1: Comparison of different marker technologies

Feature	RFLPs	RAPDs	AFLPs	Microsats	SNPs
Amount of DNA required in Micrograms	10	0.02	1.0	0.05	0.05
*Quality of DNA required	High	High	Moderate	Low/Moderate	Moderate/ High
PCR based	No	Yes	Yes	Yes	Yes
Size of the Markers	1.0 – 3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of Use	Not easy	Easy	Easy	Easy	Easy
Amenable to Automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development Cost	Low	Low	Moderate	High	High
Cost of testing per sample	High	Low	Moderate	Low/Moderate	Low/Mo derate

\* High quality DNA means that most of the DNA sequence is intact. Once a tree is felled, the DNA starts to degrade. Also, certain wood production techniques such as kiln drying degrade the DNA further.

Table 1 presents a comparison of features of the different marker technologies relevant to timber tracking.

The use of RAPD markers is not appropriate for timber tracking. Their sensitivity to laboratory conditions makes any results unreliable.

RFLPs are a time consuming and expensive technology to maintain and use, even though they are relatively cheap to develop. After the advent of PCR based markers, the use of RFLPs has been phased out.

SNPs are extremely inexpensive to maintain and run, once a unique marker (base change) has been identified for a particular tree species. They are currently unsuitable for use in timber tracking because they require the extraction of high quality DNA – difficult to extract and analyse from processed wood samples. The use of SNP markers in case of litigation to prove the origin of wood requires the matching of multiple markers.

Two main marker technologies remain - AFLP and Microsatellites (also known as Microsats) and both technologies have their own pros and cons.

The advantages of AFLPs over Microsats are that they are sensitive to the slightest genetic variations and they can be generated very simply. This variation is very useful in species diversity studies. This technique is limited to unprocessed wood however, due to the need to extract good quality DNA. Its use is thus limited to the upstream portion of the timber supply chain (up until timber is processed at the sawmill).

Microsats offer several advantages - in particular they can be extracted and analysed from degraded samples of wood. The amount of DNA required to conduct analysis is also the least amongst the different marker technologies available. The process can also be automated by using a DNA sequencer and appropriate software thereby eliminating potential human error during the comparison process. The relatively lower cost of analysis per sample is also advantageous; they consume less time and manpower compared to other technologies. On the other hand, the identification of Microsats markers is very resource intensive. For the purpose of timber tracking, it is necessary to identify at least 8 to 10 unique markers and primer combinations (A stretch of DNA that acts as an anchor point for amplifying DNA in PCR). The results using microsat techniques are very reliable; hence their use in sensitive cases such as paternity tests.

## Certisource's Technology Roadmap

The ideal DNA-based tracking solution for the timber industry is a marker technology that can successfully capture the genetic information of different species of timber as well as distinguish between intra-species variations. Markers should also be able to be extracted and analysed from processed wood samples. At the moment, no single molecular marker technique is able to address all of these requirements. Certisource is therefore pursuing research and development of multiple marker technologies, SNP-based arrays and Microsatellite techniques in a development roadmap that will ultimately enable the tracking of timber throughout the entire supply chain.

In the last two years Certisource has attained significant expertise in the application of DNA technology for timber tracking. In particular, the company has developed a process to match unique markers for the tropical hardwood *merbau* and uses this technique, together with traditional Chain of Custody processes, to verify the source and legality of this type of wood. Certisource is also engaged in research to identify markers for *kapor* and *keruing* as well as *meranti* and *balau* (also known as *bangkirai*).

Certisource is concurrently investing in Microsatellites and SNP-based array technology research for timber verification. Although some of these are at a relatively early stage and not yet ready for commercialisation, the research has already yielded several promising results encouraging further efforts.

Certisource is committed to continued research and development that will enable progressively more of the timber supply chain to benefit from DNA timber tracking, at a cost that will allow widespread adoption throughout the industry.



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