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DNA FINGERPRINTING

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ABSTRACT
DNA profiling (DNA fingerprinting) is a technique employed by forensic scientists to assist in the identification of individuals by their respective DNA profiles. DNA profiling should not be confused with full genome sequencing. Every individual in the world can be identified at the molecular level on the basis of an extremely high level of polymorphism in the sequence of his or her DNA, which he or she inherits from his or her biological parents and is identical in every cell of the body. Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different that it is possible to distinguish one individual from another, unless they are monozygotic twins. DNA profiling uses repetitive sequences that are highly variable called variable number tandem repeats (VNTRs) in particular short tandem repeats (STRs). VNTRs loci are very similar between closely related humans, but are so variable that unrelated individuals are extremely unlikely to have the same VNTRs. The application of DNA profiling in the criminal justice system is an important issue in criminal investigators today. The technology is changing rapidly and several new techniques are becoming available. DNA profiling has been described as a powerful breakthrough in forensic science. The forensic use of DNA profiling is a major contribution to a technology which can help not only in including the culprit but also to exclude the innocent.

KEYWORDS: DNA, STRs, VNTRs.

INTRODUCTION
The DNA profiling technique was first reported in 1986[1] by Sir Alec Jeffreys at the University of Leicester in England, United Kingdom[2] and is now the basis of several national DNA databases. Dr. Jeffrey's genetic fingerprinting was made commercially available in 1987, when a chemical company, Imperial Chemical Industries (ICI), started a blood testing centre in the UK.[3] Human body contains 60 trillion cells (6 x 1013). Each human diploid cell contains 23 pair of chromosomes (46) of which half is derived from each parent. Total DNA in a human haploid cell is 3 x 109 base pairs. About 99.9% of DNA code is the same for all humans. It is only rest 0.1% which is the basis of DNA profiling and all of this lies in the non-coding region. Non coding DNA (junk DNA) constitutes 97% of the total nuclear DNA while rest is coding DNA (3%).[4]

The substantial amount of non-coding DNA shows a peculiarity. It consists of large arrays (alleles) of tandem repeats of nitrogenous bases. This repetitive DNA constitutes approximately 50% of the human genome. The DNA on either side of repeats is called flanking DNA. The number of repeats is different in different person and this property is known as **tandem repeat polymorphism**[4] which is exploited in DNA profiling process.

Sample can be of two types[5]
1. Fresh un-degraded sample- As in paternity testing when fresh blood sample from putative father, mother & child is available.
2. Degraded sample- As in old dried blood or seminal stains generally found at the crime scene.

Conditions Causing DNA Degradation[5]
DNA breaks into smaller fragments when it is exposed to biological contaminants, chemicals, dirt, fungus, heat, light (sunlight or UV rays) and moisture. Their average size may become smaller than the area of interest (allele length at a particular locus). If the average size of DNA fragments in a degraded sample is smaller than the allele length, the allele would not be detected.

In degraded sample average size of DNA fragments is <500bp, thus in such a sample STR & VNTR are detectable while satellite DNA may or may not. Thus different techniques are used for fresh and degraded samples. In fresh samples - Satellite DNA & VNTR are

detected by RFLP while in degraded samples – microsatellites are detected by STR analysis using PCR.

Classification of tandem repeats[6]

1. STRs (Short tandem repeats or micro-satellites)
If the repeating unit is of the length between 1-6bps (base pairs) then the sequence is called short tandem repeats.

2. VNTRs (Variable number tandem repeats or mini-satellites): If the repeating unit is of the length between 7-100bps (base pairs) then the sequence is called variable number tandem repeats.

3. Satellite DNA: If the repeating unit is the length between 100 to several thousands of base pairs then the sequence is called satellite DNA.

DNA profiling process: The process begins with a sample of an individual's DNA called a reference sample. The most desirable method of collecting a reference sample is the use of a buccal swab, as this reduces the possibility of contamination. When this is not available (e.g. because a court order may be needed and not obtainable) other methods may need to be used to collect a sample of blood, saliva, semen, or other appropriate fluid or tissue from personal items (e.g. a toothbrush, razor) or from stored samples (e.g. banked sperm or biopsy tissue). A reference sample is then analyzed to create the individual's DNA profile using one of a number of techniques, discussed below. The DNA profile is then compared against another sample to determine whether there is a genetic match.

RFLP analysis: The first method for finding out genetics used for DNA profiling involved **RFLP analysis (Restriction fragment length polymorphism)**. DNA is collected from cells, such as a blood sample and cut into small pieces using a restriction enzyme. This generates thousands of DNA fragments of differing sizes as a consequence of variations between DNA sequences of different individuals. The fragments are then separated on the basis of size using gel electrophoresis. The separated fragments are then transferred to a nitrocellulose or nylon filter; this procedure is called a Southern blot. The DNA fragments within the blot are permanently fixed to the filter and the DNA strands are denatured. Radio-labeled probe molecules are then added that are complementary to sequences in the genome that contain repeat sequences. These repeat sequences tend to vary in length among different individuals and are called variable number tandem repeat sequences or VNTRs. The probe molecules hybridize to DNA fragments containing the repeat sequences and excess probe molecules are washed away. The blot is then exposed to an X-ray film. Fragments of DNA that have bound the probe appear as dark bands on the film.

However, the Southern blot technique is laborious, and requires large amounts of under-graded sample DNA. Also, Karl Brown's original technique looked at many mini-satellite loci at the same time, increasing the observed variability, but making it hard to discern individual alleles (and thereby precluding parental testing). These early techniques have been supplanted by PCR-based assays.

STRs analysis: The system of DNA profiling used today is based on PCR and uses short tandem repeats (STRs). This method uses highly polymorphic regions that have short repeated sequences of DNA (the most common is 4 bases repeated, but there are other lengths in use, including 3 and 5 bases). Because unrelated people almost certainly have different numbers of repeat units, STRs can be used to discriminate between unrelated individuals. These STRs loci (locations on a chromosome) are targeted with sequence-specific primers and amplified using PCR. The DNA fragments that result are then separated and detected using electrophoresis. There are two common methods of separation and detection, capillary electrophoresis (CE) and gel electrophoresis.

Each STR is polymorphic, but the number of alleles is very small. The power of STRs analysis comes from looking at multiple STRs loci simultaneously. The pattern of alleles can identify an individual quite accurately. Thus STRs analysis provides an excellent identification tool. The more STRs regions that are tested in an individual the more discriminating the test becomes.

In practice, the risk of contaminated-matching is much greater than matching a distant relative, such as contamination of a sample from nearby objects, or from left-over cells transferred from a prior test. For that reason, multiple control-samples are typically tested in order to ensure that they stayed clean, when prepared during the same period as the actual test samples. Unexpected matches (or variations) in several control-samples indicates a high probability of contamination for the actual test samples. In a relationship test, the full DNA profiles should differ (except for twins), to prove that a person was not actually matched as being related to their own DNA in another sample.

PCR analysis: Developed by Kary Mullis in 1983, a process was reported by which specific portions of the sample DNA can be amplified almost indefinitely. This has revolutionized the whole field of DNA study. The process, the polymerase chain reaction (PCR), mimics the biological process of DNA replication, but confines it to specific DNA sequences of interest. With the invention of the PCR technique, DNA profiling took huge strides forward in both discriminating power and the ability to recover information from very small (or degraded) starting samples.

PCR greatly amplifies the amounts of a specific region of DNA. In the PCR process, the DNA sample is denatured

into the separate individual polynucleotide strands through heating. PCR uses replication enzymes that are tolerant of high temperatures, such as the thermo-stable **Taq polymerase**. In this fashion, two new copies of the sequence of interest are generated. Repeated denaturation, hybridization, and extension in this fashion produce an exponentially growing number of copies of the DNA of interest. Instruments that perform thermal cycling are now readily available from commercial sources. This process can produce a million-fold or greater amplification of the desired region in 2 hours or less.

However, the PCR method was readily adaptable for analyzing VNTRs, in particular STRs loci. In recent years, research in human DNA quantitation has focused on new "real-time" quantitative PCR (qPCR) techniques. Quantitative PCR methods enable automated, precise, and high-throughput measurements. Inter-laboratory studies have demonstrated the importance of human DNA quantitation on achieving reliable interpretation of STRs typing and obtaining consistent results across laboratories.

DISCUSSION

DNA fingerprinting can conclusively prove the paternity and solve cases explicitly. Thus, with the dawn of DNA analysis and sequencing techniques, scientists increasingly began to look at people's genomes when questions of parenthood arose. DNA profiling is a tool that is not only used to apprehend the guilty but also to exonerate the innocent. As it often happens in the justice delivering system, conventional evidence can be tempered with, witnesses turn hostile, but DNA evidence remains the same. The passage of time does not affect it and neither does it change. DNA evidence thus unravels the truth - it never lies. This approach proved exceedingly useful; in fact, current marker-based methods of analysis yield test results that are both 99.99% accurate and applicable in a variety of settings. With the ongoing advancement of DNA sequencing and analytical technologies, we will no doubt continue to see an increase in the utility of these tests, as well as in the availability of detailed genetic services to the general public. Thus.

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