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Implication of DNA Source in Forensic Investigation

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ABSTRACT *DNA stands for Deoxyribonucleic acid is the fundamental building block for an individual's entire genetic make-up.The fundamental principles of DNA technology make its Significance in forensic investigation.The DNA analysis of biological evidence has been a revolutionary development in the resolution of criminal cases as genetic profile of person remain same throughout life. The scientific reason for using DNA in forensics is its unique genetic characteristics of an individual.*

Introduction

The genetic basis for forensic DNA analysis is the assay of the difference or similarity between two samples. The fundamental unit base of DNA is A, T, G, or C, and the sequence of those bases along the DNA strand[11]. A person DNA is same in every cell. For example, the DNA in a man's blood is the same as the DNA in his skin cells, hair, semen and saliva[3]. The majority of cells making up the human body are diploid cells carrying identical DNA, with the exception of haploid gametes (egg and sperm) and red blood cells (Non-nucleus). Types of biological evidence are commonly used in forensic science for the purpose of DNA analysis, including blood, saliva, semen, skin, urine and hair,etc[4].

DNA Fingerprinting:

No two human individuals (except identical twins) have exactly the same genome, this fact lead to the development of DNA fingerprinting. The technique which detect pattern of Hyper variable regions (HVRs) in DNA is known as "DNA fingerprinting" or "geneticfingerprinting"[5].Dr. Alec J Jaffreyfirst developed the technique in 1985[7].

TECHNIQUES USED IN DNA PROFILING

Restriction Fragment Length Polymorphism (RFLP):

The first forensic science applications of the technique arose from the work of Alec Jeffrey's. In 1985, the British police from West Midlands approached Jeffrey's to assist them in a rape-homicide case. Jeffrey's work resulted in the release of a wrongfully convicted man and the apprehension and conviction of the true perpetrator. Thereafter, RFLP DNA evidence contributed in forensic investigations[4].RFLP is used to analyse the different size of DNA fragments obtained as a result of DNA digestion with restriction endonuclease enzymes. This restriction endonuclease enzymes cut DNA sequence at specific sequence also called as restriction endonuclease recognition site.Resulted fragment are then separated on agarose gel by the technique known as electrophoresis and separated DNA fragments are transferred from gel to nylon membrane by known as a Southern blotting technique and location of the repeat sequences is to be established by using DNA probewhich can be labelled with radioactive isotopes(P^{32}) or with chemiluminescent dye. The labelled probes are then hybridized to the nylon membrane which emits signals on X-rays film.Normally the probes used in above techniques are of two categories i.e. Multi Locus probes and single locus probes[4, 6, and 7].

Polymerase Chain Reaction (PCR):

PCR technique, developed by Kary Mullis in 1985. It generates microgram quantities of DNA copies (upto billion copies) of desired DNA or RNA segment, present even in minute quantity in the initial preparation.The PCR amplification requires two oligonucleotide primers designed to hybridize opposite strands of the target sequence, and heat stable DNA polymerase, e.g. *Taq*(isolated from bacterium *Thermus acquaticus*),Pfu (from *Pyrococcus Furiosus*) and *Vent* (from *thermococcus litoralis*) polymerases[7, 8]. Normally PCR process follows three steps i.e. Denaturation of DNA mixture to a temperature between 90-98°C, Annealing of primer to the complementary sequences in the DNA and Primer Extension. The completion of the extension step completes the first cycle of amplification. The PCR technique is extremely useful for analysis of forensic samples, where the samples is very minute or degraded. The most popular PCR based DNA profiling techniques are Allele specific oligonucleotide (ASO)/ DNA Amplification and Amplified Fragment Length Polymorphism (AFLP)[7].

PCR Errors

Various factors can contribute to errors and inaccuracies in data produced by the polymerase chain reaction technique. PCR is often carried out using DNA polymerases such as *Taq* DNA polymerase, lacks Proof-reading ability, as a result, it commits error at a high rate (2×10^{-4})[8] .

Mispriming is also a potential problem, with products being formed from non-target sites. Excessive primer dimers may be formed, which are by-products of PCR produced when one primer is annealed to another causing primer extension[4].

Short Tandem Repeat (STR) Analysis:

Later in the 1990s, Short Tandem Repeat (STR) testing appeared in forensic DNA analysis. STR, also called microsatellites and Simple Sequence Repeats (SSRs). STRs are VNTR-like regions that have very short sequences, ranging approximately 2 to 6 base pairs (bp).The keys to the success of STR typing are multiplexing and the ability to label nucleotides with fluorescent tags.STR loci are ideal for use in forensic science for a number of reasons. They represent discrete alleles that are distinguishable from one another, they show a great power of discrimination, loci are stable in evidence samples and a small amount of sample is required due to the short length of STRs. Multiplex systems are complex and loci are selected based on their suitability in a multiplex system and their discriminating power.

Microsatellite polymorphism are now used for a wide range of applications in genetics including construction of genetic linkage map, linkage mapping of quantitative traits and disease gene, diagnosis of genetic disorders, paternity testing, for the selective breeding in the livestock, wildlife conservation , population studies and forensic studies[4] .

Variable Number Tandem Repeats (VNTRs):

VNTRs also called Minisatellites, are similar to STRs,but the repeating unit is larger than that for STRs,from 7-10 bp long.

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VNTRs were first discovered by Alec J.Jaffreys in 1985. There are two types of VNTR loci: unique loci and multicopy loci. In others words, there may be only one copy of a VNTR locus in an individual genome, or there may be a number of copies scattered around the genome [4] .These tandem repeats are inherited from both parents, therefore no one will have the same VNTRs as either of their parents.

Low Copy Number (LCN) Analysis:
Low Copy Number Analysis, is a technique developed by the UK's Forensic Science Service in an attempt to increase the sensitivity of DNA profiling methods. Samples containing small amounts for badly degraded DNA often leads to problems such as poor quality fingerprints or even completely negative results. This technique reduced these issues. Developed in 1999, LCN is essentially an extension of the Second Generation Multiplex Plus technique. Improved result is achieved through an increased number of PCR cycles, with standard techniques generally using 28 cycles but LCN using 34. This could ultimately allow for DNA profiles to be successfully obtained from minute amount of sample[4].

Single Cell DNA Fingerprinting:
Dr Ian Findlay and his colleagues at the Australian Genome Research Facility first reported the successful development of a DNA fingerprint from a single cell in 1997. The single cell is obtained by swabbing the material and identifying the cell to be analysed using microscopy prior to analysis. This technique is particularly fast, taking a matter of hours. Single-cell DNA profiling is particularly useful in rape cases, as DNA in sperm cells is highly conserved due to it being so compacted in the protein head. There is also potential for the technique in use in documents. Human DNA can be placed in documents such as Government bonds, wills and security documents, to track their flow. However the main issue with this particular use is that close relatives may handle the documents, particularly when dealing with documents such as wills, and so the technique may not be appropriate[4].

Mitochondrial DNA (mtDNA) Analysis:
Mitochondrial DNA is a circular molecule of DNA 16,569 base pairs in size, first referred to as the Anderson sequence, obtained from the mitochondrion organelle found within cells[4]. This sequence is entirely functional and highly conserved, so there is very little variation between individuals. However there is a 1000 base pair long non-coding D-loop, known as the control region, which contains two hypervariable regions referred to as HV1 and HV2. The variations within these regions are single nucleotide polymorphisms (SNPs) and it is the regions that are focused in the forensic analysis of mtDNA [4]. The most significant advantage of the use of mitochondrial DNA is the possibility of analysing even highly degraded, burnt or old samples and hair without root, which cannot yield any results with nuclear DNA analysis, mtDNA can generate a profile due to protection provided by its ring structure and its high quantity[1, 9.] As mtDNA is only maternally inherited,thus all generations springing from the same mother carry the same mtDNA characteristics, this cannot form a full DNA fingerprint of the individual, thus this technique is only beneficial if the DNA profiles of maternal relatives are available, such as the individual's mother or biological siblings.

Y- Chromosome Analysis:
One particular branch of DNA analysis focuses on the amelogenin marker, the only marker on the sex chromosome, useful in the analysis of the Y chromosome. The Y chromosome, generally found only in males. However similar to mtDNA (which is maternally inherited), the combination of alleles in this instance is theoretically identical between father and son, assuming mutation does not occur. Y chromosome analysis is particularly useful in cases of sexual assault and rape in which mixed DNA profiles may be encountered. Numerous systems have been developed to analyse some of the STRs present on this chromosome, such as Applied Biosystems' Yfiler. The DNA analysis defined as Y-STR is particularly helpful in the resolution of rape cases, paternity cases[4].

Touch DNA:
One such technology is called "Touch DNA" or "Contact Trace DNA." Touch DNA refers to the DNA that is recovered from skin (epithelial) cells that is left behind when a person touches or comes into contact with items such as clothes, a weapon, or other objects. A person lower skin cells will provide the best DNA profile. These cells are typically recovered when force is used such as on the victim's clothes or at a crime scene after a struggle has occurred. These epithelial cells can be lifted with a tape, swabbed with a Q-tip, or even scraped from the clothes of the victim, or objects [10].

Discussion:
The aim of paper try to reveal the importance of DNA and techniques used and its sensitivity in Forensic science, were most of the incidents resolved by DNA analysis i.e. the identification of disaster victims. Natural disasters, Mass disasters, Fire, Transport accidents, Terror event are defined as disaster. In events like these, the place and time of which cannot be foreseen, many people die at the same time and their bodies generally change beyond recognition. Identification of the dead as soon as possible and in an accurate manner is vital from the human, religious, social and legal points of view.This technique is mainly focussed in research area, as DNA polymorphism at a very large number of loci makes each genome almost unique. DNA analysis established the guilt of suspect or already convicted individual innocent.

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