A STUDY ON APPLICATION OF DNA IN FORENSICS

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ABSTRACT

The technique of finding out variations for identification of individuals of a population at DNA level is termed as DNA FINGERPRINTING. It works on the principle of polymorphism in DNA sequences. Since, the DNA from every tissue such as skin, semen, saliva, swabs, hair follicle, bone or any nucleated cell confer the same degree of polymorphism and thus contribute to a very useful identification tool in forensic applications. In this study DNA isolation is done from molar tooth and its application in forensics is evaluated in DNA bands. The isolated DNA samples contribute a lot in resolving criminal cases at scientific level.

Key Words: DNA Bands, DNA Isolation, DNA Fingerpritting.

I.INTRODUCTION

DNA known as deoxy ribo nucleic acid is the genetic material, at least for the majority of organisms. It is a polymer of nucleotide repeats which are linked through high energy phosphodiester bonds in a poly nucleotide chain. Every living organism is made up of cells and the cells contain DNA. However some viruses like TMV, HIV contain ribonucleic acid (RNA) as genetic material. Like most eukaryotes, humans possess DNA in their cells as genetic material and the haploid content of human DNA is almost 3.3×10^9 Base pairs.

DNA an acidic substance present in the nucleus of a cell was first identified by friedrich meischer in 1869 and he named it as nuclein .However, due to technical limitations in isolating such a long polymer of nucleotides intact, the understanding of this molecule of life remained elusive until two nobel laureates james Watson and francis crick in 1953 proposed the double helical structure of the DNA. One of the hallmarks of their proposition was pairing of purine bases (adenine, guanine) with pyrimidines (cytosine ,Thymine) in two DNA strands . the base pairing confers a unique property to the poly nucleotide chains where in adenine in one strand forms two hydrogen bonds with thymine in the other strand and guanine in one strand forms three hydrogen bonds with cytosine in the other strand and vice versa . The two DNA strands are complementary to each other i.e if base sequence of one strand is known, the sequence of bases in the other strand can be predicted.

In a typical mammalian cell the length of DNA is approximately 2.2 meters which is in compaction with basic proteins called histones in micro meter dimension of a nucleus. The negatively charged DNA is wrapped around positively charged histones H2A, H2B, H3, and H4 in an octamer called as nucleosome. A typical nucleosome contains 200 base pairs of DNA helix. The nucleosomes constitute repeating unit structure in nucleus called chromatin .when viewed under electron microscope, the nucleosomes appear as beads on a string .

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The first use of DNA test in forensics as forensic evidence came in 1986 where two young girls Lynda Mann and Don Aishworth were sexually assaulted and then left brutally murdered in 1983 and 1986 respectively . Both the murders were committed in the same manner and the investigation team suspected that the same man had committed the crimes. A local man volunteer to the confession for having committed the crime tested negative for the profile generated from his donated blood. The DNA profile of him didn't match the profile generated from the semen stains recovered from the scene of crime. A year later a woman at a bar in England overheard someone bragging about how he had given a blood sample for a friend named as Colin pitch fork who was later found guilty of committing the crimes as the profile generated from his blood matched with the profile generated from the semen stains recovered from the crime scene . This technique of finding out variations for identification of individuals of a population at DNA level is termed as DNA FINGERPRINTING. It works on the principle of polymorphism in DNA sequences. since, the DNA from every tissue such as skin, semen, saliva, swabs, hair follicle, bone or any nucleated cell confer the same degree of polymorphism and thus contribute to a very useful identification tool in forensic applications. The degree of polymorphism (variation at genetic level) is inheritable from parents to children as the offspring receives his genome from both the parents' semi conservatively. DNA fingerprinting technique also called as DNA typing or Genetic profiling was invented by an English geneticist sir Alec Jeffery's in 1984 at Leicester university, united kingdom. Sir Alec jaffery's found that certain regions of DNA contained sequences that were repeated over and over again next to each other. He also discovered that number of repeated sequences present in a sample could differ from individual to individual. DNA FINGERPRINTING involves extraction of DNA molecules from the biological samples, subjected to quantisation and amplification in a polymerase chain reaction set up and later followed by short tandem repeat (STR) analysis. STR locus or position is a short DNA sequence repeated many times in tandem at particular location in a chromosome. Since each human inherits one chromosome from each parent, the STR lengths on the two chromosomes are often different generating two signals from one individual. If multiple STR loci are analyzed, a profile can be generated that is essentially unique to a particular individual. Short tandem repeats (STRs) are preferred genetic markers for personal identification due to their abundance throughout the genome, population specific variability and small size, compatible with the degraded DNA samples.

In order to generate the genetic profile in the populations of central India , the analysis of 15 autosomal STR loci is done , however in October 1998 FBI launched USA national DNA INDEX SYSTEM (NDIS) including 13 core STR loci (TH01 , v WA , FGA, TPOX, CSF1PO , D8S1179, D18S51, D21S11, D3S1358, D5S818, D7S820, DI3S317, D16S539).

II.MATERIALS AND METHODS

Extraction of DNA from Tooth sample. Method used is organic extraction method.

Extraction protocol

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- A non restored tooth usually molar one is the sample of choice for extraction of DNA however, any tooth sample can yield the amplifiable DNA for genotyping.
- pulverize the tooth and crush the sample to a powdered form
- place the processed tooth sample into a 1.5 ml eppendorf tube and to the sample add 300 µl of stain extraction buffer + dithiothreitol (0.006gm/ml of SEB) 20 micro litres of proteinase K. vortox for 10 seconds and incubate at 56 degree Celsius in water bath for two days.
- Add 400 μ litres of phenol : chloroform: isoamyl alcohol (25:24:1); vortex for 30seconds, centrifuge at 15000rpm for 15 minutes.
- Carefully remove the aqueous layer (top most layer) place in a fresh 1.5 ml of eppendorf tube
- To the aqueous layer add 1.0 ml cold absolute ethyl alcohol. Mix by inversion of the tube. Place the tube at -20 degree Celsius for 15 minutes. centrifuge the the content at 10,000 rpm for 10 minutes
- Remove and discard the supernatant by decantation.
- Wash the pellet with 70% ethanol. centrifuge at 10,000rpm for 10 minutes
- Air dry
- DNA ready for quantitation.



PROCESSING OF TOOTH SAMPLE FOR DNA EXTRACTION USING TOOTH CRUSHER

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> CRUSHED TOOTH SAMPLE IN FALCON TUBE

BANDS OF EXTRACTED DNA IN AGAROSE



Figures showing experimental work during DNA extraction and Loading

III. RESULTS AND CONCLUSIONS

PROSPECT OF DNA PROFILING IN FORENSICS

The crime gazette of jammu and kashmir for the previous two years has depicted an appreciable increase or positive fluctuation in various crime heads. 57 cases of murders were reported in the year 2016 and increased to the no. Of 77 cases in 2017. More over, 98 females complained of sexual assault in 2016 and 159 rape cases were registered in 2017. Timely justice to the victims of crime or their families largely depends on scientific evidence wherein the DNA FINGERPRINTING has a major role to play in speedy disposal of criminal cases so that to administer pure justice in the societal setup.

DNA fingerprinting has multiplex role in no. Of cases including:

- Maternity and paternity disputes
- Identification of rapist(s)
- Identification of mutilated remains of the body of deceased
- Identification of missing child .
- Child swapping cases in hospitals
- Linking the suspect to the biological evidence found at the crime scenes in murder cases .
- Identification of mass disaster victims
- Poaching in wild life.

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