Role of Short Tandem Repeats from trace DNA in Forensic Casework

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Abstract:-

Over the past twenty years, DNA analysis has been revolutionalized forensic science and has become a dominant tool in law enforcement. The DNA is unique for each and every person. This application is used in forensic science to identify the criminals. The DNA is isolated from the biological samples collected from the crime scene. DNA can be extracted from saliva, blood, hairs, semen or any body fluid. The various materials like cigarette, chewing gum or any material containing body fluids may contain DNA as it is used by the person. The person is then identified by various methods like STR, RFLP, and VNTR. The identification of person is done by STR and is most applied method in forensic science.

As the relevance and value of DNA profiling to forensic investigations has increased, so too has the desire to generate this information from smaller amounts of DNA. Trace DNA samples may be defined as any sample which falls below recommended thresholds at any stage of the analysis, from sample detection through to profile interpretation, and cannot be defined by a precise picogram amount. In this case here our aspects associated with the collection, DNA extraction, amplification, profiling and interpretation of trace DNA samples. Many times blood grouping does not reveals any group from trace biological evidences collected from the crime scene. The analysis of short tandem repeat (STR) DNA sequences from trace quantity of DNA is of fundamental importance to forensic science to solve the difficult criminal cases.

Keywords:- trace DNA, short tandem repeats, forensic identification.

Introduction:-

Forensic DNA typing currently relies on a number of well-defined autosomal short tandem repeat (STR) markers and analysis by capillary electrophoresis (CE) [1-3]. Even with reasonably large multiplexes (reaching up to 27 markers per kit) and their associated high discrimination power, there are certain scenarios, such as complex mixtures and some challenging kinship cases, in which these standard DNA typing approaches are insufficient [4-7].

DNA forensic is the bed rock tool for crime investigation. It is one of the very accurate and reliable measures that have become the main source in the law enforcement. The DNA is being fundamental structure of the life and always unique for the individual. 99.99% of the human genome is similar and contains exons and introns. Only 0.1% of the DNA is different and gives the specificity to each person. During any case investigation various physical, testimonial and biological kinds of evidences may be found at the crime scene.[8] Out of these biological evidences are very important. These include saliva, blood, semen or any biological fluid. These fluids contains variable amount of DNA and can ensure the CSIOs that the source person of DNA was present at the crime scene. However it doesn't confirm the person as the culprit or his direct involvement in the crime, but he can't refuse his presence on the crime scene. DNA forensic involves collection of samples with proper care and fingerprinting of DNA. The various techniques in the DNA forensic include PCR, STR, VNTR and RFLP etc. Restriction Enzyme Length polymorphism and Short Tandem Repeats gives the band pattern on the gel which is very specific. Using these technologies the individual can be identified. The DNA forensic is also used in the paternity identification and the missing cases. The qualification of DNA can be done by the Agarose gel electrophoresis method.[9] A major improvement that the forensic community has developed to assist in the typing of degraded and trace DNA has been the recent re-engineering of primers of the core STR systems, such that they are positioned closer to the repeat units in order to reduce the
length of the amplified flanking regions [10-20]. The use of mobility-shifters (non-nucleotide linkers, Applied Biosystems, CA, USA) and novel multiplex design strategies [such as Promega's ESI and ESX kits (WI,USA)] have allowed all the core CODIS (Combined DNA Index Systems) and European set of loci to be amplified as 'miniSTR' loci, thus increasing the profiling success rate from difficult samples [19,21].

Case History:-

In this case a person was filing a complaint about missing of his brother. Police officials tried to find the missed person from last month but they didn't get any success to find him. After one month of period a body which was found decomposed state in the well at unknown place. Relatives identified the body. After post mortem from the head injuries it was clear that the deceased was first killed and then thrown in to the well.

The police persons arrested some suspects after investigation five accused were found culprit in that. But due to the lack of evidence it was very difficult to prove murder. When police officials interrogate the accused; they told that first they used a metallic pipe to injured the deceased and after death they carried his body though the motor car and thrown in to the well filled with water at unknown place. And to hide the evidences they washed motor car, burnt the cloth articles of accused and thrown the weapon (metallic pipe).

After one month of crime scene we found the traces of blood on seat cover & mat of the motor car used in the crime, blood mixed earth, pair of chappal, small cloth piece, hairs found at crime scene and weapon (metallic pipe) removed from the well filled with water.

In this case it was so difficult to tally the blood group of deceased and also to do the DNA fingerprinting from such trace quantity of biological evidences. But by using different methods for isolation of DNA we

got the success to retrieve the trace quantity of DNA from traces of blood on seat cover & mat of the motor car, blood mixed earth at crime scene, pair of chappal at crime scene and weapon (metallic pipe) removed from the well filled with water after one month. After DNA fingerprinting we came to conclusion that the DNA retrieved from such evidences tallies with the DNA of sternum bone of the Deceased.

Materials & Methods:-

Materials: Table 1

Reagents	**Parameters**
Forensic Buffer	1 ml Tris HCL-100ml 0.5ml EDTA Buffer -10ml 5M Nacl-10ml Make the volume upto 1000ml
Proteinase K	Appearance- Colorless solution in 50% glycerol, cont.20mM Tris.,1mM $Cacl_2$,PH ca.7.4 Concentration 20mg solid/ml
Investigator kit	Buffer G2,Prot.K,Carrier RNA,
AmpFSTR Identifiler®PCR amplification Kit	Allelic Ladder,AmpliTaq Gold®DNA polymerase, Primers,
Hi-Di™Formamide	CAS 75-12-7,CAS 60-00-4
Size Standard	GeneScan™-500,LIZ ™

Table-2 EZ1 Automate DNA Extraction System Parameters

Instrument	**Operating Parameters**
Kits designed for this instrument	QIAGEN EZ1 Kits
Pipetting range	50-1000 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
Samples per run; throughput	6 samples per run
Technical data of the instrument	Weight 48 kg, 100–240 V AC, 50–60 Hz
Technology	Magnetic-particle technology

Table-3 Automate Express DNA Extraction System Parameters

Instrument	Operating Parameters
Kits designed for this instrument	Prep Filer Express
Pipetting range	20 µL to 250 µL
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
Samples per run; throughput	13 samples per run
Technical data of the instrument	Weight 51kg, 240 VA, 50⁄60 Hz, Grounding is necessary, Required input power AC 100-240 V ±10%
Technology	Magnetic-particle technology

Table-4 PCR Thermal Cycler Machine

Instrument	Operating Parameters
Capacity	96 wellx0.2ml PCR tubes/one 96 well plate
Heating/cooling	Peltier based
Capable of testing temperatures	Denaturation, Annealing & Extension steps
Block ramp rate	5.0ºC/Sec.
Sample ramp rate	4.4ºC/S
Temperature range	4-99ºC/S
Temperature accuracy	±0.2ºC
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

Table-5 Genetic Analyser-3500

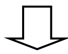
Instrument	Operating Parameters
Fragment Size(bp)	500bp
No. of Markers	16
Polymer	POP4
Detector	CCD
Oven Temp	60ºc

Column Size	36cm
Software	GeneMapper®

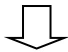
Method: Ez1

Extraction of DNA from Sternum bone piece, prepared blood stain from blood detected on exhibits like piece of set cover , earth, pair of chappal, small cloth piece , hair , hollow pipe

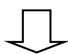
Carefully clean the platform of workstation of laminar flow with ethyl alcohol



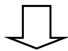
Take approx 0.5 mm sample piece and cut respective sample into small pieces and take into 2ml micro-centrifuge sample tube



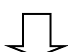
To this Add 400µl Forensic Buffer +25 µl Protease K+40 µl 1mM DTT



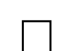
Vertex and spin



Incubate at 56˚C overnight on Thermo-Shaker



To the next day, transfer the sample into the EZ1 micro-centrifuge sample tube



Set the micro-centrifuge sample tube in the EZ1 Advanced (Quiagen) magnetic bead based liquid handling system for automate DNA

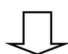
Isolation

Store the extracted DNA at -20˚C

Method:-Automate Express

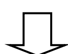
Extraction of DNA from prepared blood stain from blood detected on exhibits like piece of set cover , earth, pair of chappal, small cloth piece , hair , hollow pipe

Take 0.5 mm sample+ 220 µL Lysis Buffer +3 µL freshly prepared 1 M DTT + 7 µL Proteinase K in micro-centrifuge Tube

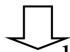


Tightly close the lid of the column/tube assembly. Do not place labels on tube caps; doing so can cause leakage

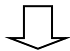
Place the column/tube assembly in a thermal shaker, and then incubate it at 56°C and 750 rpm for 40 minutes



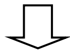
Centrifuge the column/tube assembly for 2 minutes at 10,000 × g to transfer the lysate to the sample tube.



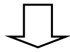
Carefully remove the column from the sample tube. If there is clear lysate remaining in the column, transfer the lysate to the sample tube.



Load the sample tube into the Automate Express Machine and start the process



After 40 min DNA eluted into the Elution tube



Remove the elution tube and store the DNA for at -20˚C

Polymerase Chain Reaction:-

Reagents Volume

PCR reaction Mix -10.5 ul

Primer set- 5.25 ul

Sample input -10 ul

Table 6 PCR

Step	Ampli Taq Gold Enzyme Activation	PCR			PCR Final Step	PCR product till separation of STRs
	Hold	CYCLE (28 cycles)			Hold	
		Denaturation	Annealing	Extention		
Temp	95˚C	94 ˚C	59 ˚C	72 ˚C	60 ˚C	4 ˚C
Time	11 min	1 min	1 min	1 min	60 min	∞

Genotyping: -

STR genotyping is detected and analysed on 3500 Genetic Analyser (Applied Biosystems) instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.

Sample Preparation for Injection

Standard Mix

1µLSingle or pooled PCR product

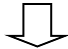
0.5 µLSize standard (for GeneScan500-LIZ®only 0.3 µL)

10-20 µLHi-Di™ formamide (PN 4311320)

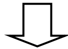
Denaturation of PCR product.

(90 –95 °C, 2 –5 min)

Immediately on ice or cool to 4 °C in thermal cyclerLoad the mixture in auto sampler on instrument for injection.



Electrophoresis is done through fine glass capillary filled with polymeric gel.(During capillary electrophoresis, the extension products of the PCR reaction and any other negatively charged molecules such as salt or unincorporated primers and nucleotides) enter the capillary as a result of electro kinetic injection. The extension products are separated by size based on their total charge)



DNA fragments travel through capillary according to their size & reach the window which coincides with the Laser device in the instrument.(Shortly before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size, move across the path of a laser beam. The laser beam causes the dyes attached to the fragments to fluoresce.)

Laser excites the fluorescently labelled DNA fragments.(The laser beam causes the dyes attached to the fragments to fluoresce.)

CCD Camera behind the window records the excitation peaks.

(The dye signals are separated by a diffraction system, and a CCD camera detects the fluorescence.)

Excitation peaks for 16 different loci are obtained.(Because each dye emits light at a different wavelength when excited by the laser, all colors, and therefore loci, can be detected and distinguished in one capillary injection.)

For each set of sample standard allelic ladder is run.

Data Collection software collects the data of these excitation peaks.

(The fluorescence signal is converted into digital data, and then the data is stored in a file format compatible with an analysis software application.)

Results:-

The DNA extracted from,

1) Sternum bone of Deceased

2) Prepared blood stain from blood detected on piece of set cover and Mat of motor car

3) Prepared blood stain blood detected in earth, pair of chappal, small cloth piece, hair and hollow pipe from

was typed at 15 STR loci and gender specific Amelogenin locus using PCR amplification technique.

Table 7 -The results of DNA typing is summarized below:-

STR	Genotype							
	piece of seat cover of car (Crime Scene)	piece of Mat of car (Crime Scene)	Earth (Crime Scene)	Small cloth piece (Crime Scene)	Hairs (Crime Scene)	Pair of chappal (Crime Scene)	Hollow Metallic pipe (Weapon)	Sternum bone Deceased
D8S1179	12,12	12,12	12,12	12,12	12,12	12,12	12,12	12,12
D21S11	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31
D7S820	8,11	8,11	8,11	8,11	8,11	8,11	8,11	8,11
CSF1PO	9,11	9,11	9,11	9,11	9,11	9,11	9,11	9,11
D3S1358	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
THO1	7,8	7,8	7,8	7,8	7,8	7,8	7,8	7,8
D13S317	12,12	12,12	12,12	12,12	12,12	12,12	12,12	12,12
D16S539	10,13	10,13	10,13	10,13	10,13	10,13	10,13	10,13
D2S1338	16,22	16,22	16,22	16,22	16,22	16,22	16,22	16,22
D19S433	13,13	13,13	13,13	13,13	13,13	13,13	13,13	13,13
vWA	16,18	16,18	16,18	16,18	16,18	16,18	16,18	16,18
TPOX	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11
D18S51	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13
FGA	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23

Table No.6 shows DNA profiles obtained from blood detected on piece of seat cover & piece of mat of motor car, earth, small cloth piece, pair of chappal, hairs found at scene of crime and weapon hollow metallic pipe by accused **matched** with DNA profile obtained from sternum bone of Deceased and also are identical and from one and the same source of male origin.

Discussion:-

Throughout the years of trace DNA use, the major focus has been on improving techniques in order to obtain highly discriminating genetic profiles from minute amounts to help identify the person from whom the DNA at a crime scene is derived. However, much less effort has been expended on understanding the activities that explain how the DNA got there.

DNA forensics technique is highly authentic and very much accurate. Each person has similarities and differences in DNA sequences. DNA forensic works on the basic like extraction, isolation and identification. The source person of the DNA can be identified by using RFLP and STR. DNA can be

isolated from the various materials like cigarette, lipstick print or chewing gum used by the person. The quantity of the DNA however differs. By above studies and results, we can conclude that, the evidences concern with direct contact with saliva like chewing gum are most important as they can provide considerable amount of DNA. Such samples should be collected with prime priority and preserved.

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