JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

STR TYPING: BIOLOGICAL PROBE FOR IDENTIFICATION OF CRIMINALS IN FORENSICS

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Abstract

Short tandem repeat (STR) typing continues to be the primary workhorse in forensic DNA profiling. Therefore, the present review discusses the prominent role of STR marker in criminal justice system. All overthe world, deoxyribonucleic acid (DNA) profiling provides evidence that may be used to convict criminals, as an irrefutable proof of wrongful convictions, invaluable links to the actual perpetrators of crimes, and could also deter some offenders from committing more serious offences. Clearly, DNA profiling tools have also aided forensic scientists to re-evaluate old cases that were considered closed as a result of inadequate evidence.

Use Short Tandem repeats in forensic casework is become an important tool to reach the real culprit in criminal case like rape, murder, robbery etc. Most of the time due to the lack of circumstance evidences in judicial system becomes a strong benefit to the accused. In Maharashtra day by day rate of heinous crimes and lot of rape crimes increased. In rape case medical examination sampling by medical officer play an important role to avoid the loss of trace biological evidence. In sexual offences, specifically semen on victim's clothes or biological samples proves involvement of accused in the crime. But in some sexual offence cases where contraceptive devices are used or semen stains are not detected during investigation, the victim's blood or body fluid on the accuser's garments or vice versa helps to prove the rape crime. Most of the times ABO grouping can give the results of specific group but still due to similarities in blood groups we cannot make a conclusion about involvement of any specific person in sexual offence case. Here, ABO grouping as well as DNA profiling technique has created wonders from the time it has been invented. Once the DNA technique proves involvement of the accused, there is provision of 'Protection of Children from Sexual Offences' (POCSO) Act 2012 in the court to effectively address the heinous crimes. In the instant case, a girl aged 9, promising to give chocolates by minor accused, sited her on motor cycle and was raped in farm. During rape the accused private part becomes injured and his blood transferred on undergarments and cloths of the victim. As the DNA profiling proved his involvement in the crime.

Keywords:- Forensics, Rape Assault, PCR,STR,DNA profiling

Introduction

Recently sexual offences against women and number of minor victims have been increase day by day. Census data from 2011 shows that in India 472 million children below age of eighteen and out of them 225 million are girls [1]. Due to pressure and lack of knowledge, these children fear to talk about atrocious crime. India has taken lot of precautions since the 'Nirbhaya' case happened and made legal provision of new act "Protection of Children from Sexual Offences Act

2012 (POCSO Act 2012)" and continuously monitoring these cases [2] It is difficult to identify a criminal just by examining the trace of blood on crime scene and the garments. In sexual offences, a specifically biological fluid on victim's clothes proves involvement of accused in the crime. But in some cases where contraceptive devices are used or semen stains are not detected during investigation, the victim's blood or body fluid on the accuser's garments helps to prove the crime. It is difficult to prove the evidence by routine ABO grouping in most of the cases because of the less quantity of blood. Further, discrimination power of ABO Blood group system is less. Here, DNA profiling technique has created wonders from the time it has been invented. Once the DNA technique proves involvement of the accused, there is provision of 'Protection of Children from Sexual Offences' (POCSO) Act 2012 in the court to effectively address the heinous crimes.[3]. While performing forensic analysis of exhibits seized in sexual offences, though semen is absent in medical samples like vaginal swab, pubic hair or on her clothes, if blood of accused is found on her exhibits, it can play a vital evidence to prove the crime. This evidence of blood is very much important in specifically minor victims. Because in such crimes, it is observed that most of the times, accused fails to intercourse and ejaculate semen because of smaller opening of vagina of minor girl and he himself gets penile injuries while forceful attempt. Blood detected in such cases is important to prove the crime [4,5]

Blood grouping technique is widely used in forensic laboratories for investigation of biological fluids collected from crime scenes. In 1900, Sir Karl Landsteiner discovered the blood grouping technique known as the "ABO" system for which he was awarded with Nobel Prize in 1930 [6]. Edmond Locard a pioneer of forensic science proposed that every criminal carries some trace of evidence with him or her from the scene of crime by which he or she can be linked with the crime. The strongest evidence from the crime scene available is blood or blood stains because the source of blood and their stains help in solving the crime of violence, sexual offences, vehicular accident cases or murder. In cases of natural disasters the prime identification of body part is ABO blood grouping then later comes DNA matching. This is the most commonly followed techniques in today's forensic laboratory analysis [7,8,9]

DNA profiling in forensic science in the UK is focused on the analysis of short tandem repeat (STR) loci using PCR. It is the technique of choice for the national strategy to create criminal intelligence databases. Apart from the increased sensitivity inherent with any PCR technique, with STRs there is also the advantage of definitive allelic identification. This is a consequence of lower measurement errors associated with the use of polyacrylamide gel electrophoresis to detect DNA fragments ranging between 200-400 bp in size [10,11,12]

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Materials and Methods:

Short tandem repeat (STR) typing methods are widely used today for human identity testing applications including forensic DNA analysis. Following multiplex PCR amplification, DNA samples containing the length-variant STR alleles are typically separated by capillary electrophoresis and genotyped by comparison to an allelic ladder supplied with a commercial kit. This works provide a brief perspective on the technologies and issues involved in STR typing.

Materials-

Table 1 : Reagents and Chemicals Reagents

Reagents and Chemicals Reagents	Parameters
Forensic Buffer	1 ml Tris HCL-100ml 0.5ml 0.5ml EDTA Buffer -10ml 5M,Nacl- 10ml Make up volume
Proteinase K	Appearance- Colorless solution in 50% glycerol, cont.20mM Tris., 1mM CaCl2, PH ca.7.4 Concentration 20mg solid/ml
Investigator kit Amp FSTR Identifier® PCR amplification Kit	Buffer G2, Prot. K, Carrier RNA, Allelic Ladder, AmpliTaq Gold® DNA polymerase, Primers,
Hi-DiTMFormamide	CAS 75-12-7, CAS 60-00-4
Size Standard	GeneScanTM-500, LIZ TM

Table-2:EZ1 Automate DNA Extraction System

Instrument Operating	Parameters
Kits designed for this instrument	QIAGEN EZ1 Kits
Pipeting range	50-1000 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
DNA Samples per run	Throughput 6 samples per run
Technology	Magnetic-particle technology

Table-3: Polymerase Chain Reaction 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	5.0°C/Sec. Sample ramp rate 4.4°C/S
Temperature range	4-99°C/S
Temperature accuracy	±0.2oC
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen,about 8.5 in

Steps used in analysis:-

Detection of Biological fluids:-

In this case, we received different cloth articles collected by investigation officer and medical samples like vaginal swabs, pubic hair, nail clippings and reference blood sample of victim and hair, penile swab, nail clippings, reference blood sample of accused. While analysis, the blood was detected on frock, knicker of victim, Neither blood nor was semen detected on medical samples of both. Routine Kastle -Meyer solution was used for detection of blood. Semen was tested by using acid Phosphatase test.

Extraction of DNA:-

DNA was extracted from blood detected on frock, knicker of victim and full jeans pant, underwear of accused. The DNA extraction was done using Automate Express machine using Prep Filer™ Express DNA extraction kit. The Prep Filer ™ Forensic DNA extraction Kit (Applied Biosystems, Foster City, CA) is efficient for isolation of DNA from a variety of biological samples that contain small quantities of biological material so that if traces of undetected are present in blood on cloths or medical samples.

The protocol used for extraction was as follows:-

- Blood stains on all the positive articles were cut into small 1 x 1 mm pieces and were placed in 2ml micro centrifuge tube.
- 500 µlLysis buffer from PrepFiler Express F DNA extraction kit (19) was added to all the sample tubes.
- The sample tubes were kept on thermo shaker at 750 rpm at 70 ˚C for 40 min [15].
- The tubes were then centrifuged at 10,000 rpm for 2 min [16].
- Cartridges from PrepFiler Express F DNA extraction kit were loaded to the cartridge rack in Automate Express DNA extraction system (20), Sample tubes, elution tubes and tips were loaded as per machine guidelines and the machine program was run as per the recommended machine protocol.
- After completion of program, elution tubes containing extracted DNA in highly pure form were stored at 4˚C till the next PCR amplification process.
- Different methods are available for extraction of DNA. This organic extraction method was employed for extraction of DNA from reference blood samples of accused and victim in both the cases. In organic extraction method, samples were lysed using Forensic Buffer (pH 8), Proteinase K, and Sodium Dodecyl Sulphate. Further samples were incubated for 2 hrs at 56˚C and Phenol: Chloroform: Isoamyl alcohol previously prepared solution was added.
- The aqueous layer containing DNA separated and treated with 2 M Sodium Acetate and the DNA was precipitated using chilled Isopropanol.
- Finally extracted DNA dissolved in TE buffer (pH 7).

Quantification of DNA:-

Extracted DNA was quantified using Quantifiler human DNA kit on 7500 Real Time PCR System (Applied Biosystems) according to the protocol. Proper diluted DNA sample was used for further PCR reaction. Master mix used for Polymerase Chain Reaction was AmpFISTR PCR reaction mix: 10.5 µl AmpFISTR Primer Set: 5.5 µl Polymerase: 0.55 µl Volume of Master mix used: 15 µl Volume of DNA sample: 10 µl After PCR amplification denaturation was carried out using HiDi Formamide and Liz 600 size Standard.

STR Genotyping:-

After completion of PCR amplification of DNA, amplified DNA products were analyzed on 3500 Genetic Analyzer and processed using Gene Mapper® ID-X Software V 1.5 according to manufacturer recommended procedure. Simultaneous amplification of 16 STR Loci was achieved.DNA profiles obtained from above samples in both cases were interpreted and compared with each other.

Results and Discussion:-

The DNA extracted from exhibit prepared blood stains of accused and victim, blood stain cuttings from knicker, frock of victim and full jean pant, underwear of accused was typed at 15 STR Loci and gender specific Amelogenin locus using PCR amplification technique.

The Results of DNA Typing are summarized as follows.

STR LOCUS	GENOTYPE					
	DNA of blood stain knicker of victim	**DNA of blood stain frock of victim**	**DNA of blood stain full jean pant of accused**	**DNA of blood stain underwear of accused**	**DNA of reference blood stain of victim**	**DNA of reference blood stain of accused**
D8S1179	12,13	12,13	12,13	12,13	11,15	12,13
D21S11	33.2,33.2	33.2,33.2	33.2,33.2	33.2,33.2	29,30	33.2,33.2
D7S820	10,11	10,11	10,11	10,11	8,11	10,11
CSF1PO	10,11	10,11	10,11	10,11	12,12	10,11
D3S1358	15,16	15,16	15,16	15,16	15,16	15,16
TH01	8,8	8,8	8,8	8,8	6,9	8,8
D13S317	8,12	8,12	8,12	8,12	8,13	8,12
D16S539	9,12	9,12	9,12	9,12	11,11	9,12
D2S1338	23,23	23,23	23,23	23,23	18,22	23,23
D19S433	12,14.2	12,14.2	12,14.2	12,14.2	13,14	12,14.2
vWA	17,17	17,17	17,17	17,17	16,17	17,17
TPOX	8,11	8,11	8,11	8,11	8,8	8,11
D18S51	11,14	11,14	11,14	11,14	12,14	11,14
AMEL	X,Y	X,Y	X,Y	X,Y	X,X	X,Y
D5S818	10,11	10,11	10,11	10,11	10,11	10,11
FGA	22,22	22,22	22,22	22,22	19,23	22,22

Interpretation:-

DNA profile obtained from blood stain cuttings from knicker, frock of victim and full jean pant and underwear of accused are **identica**l and from one and the same source of male origin and **matched** with DNA profile obtained from reference blood stain of accused.

Conclusion:-

The amount of DNA required for analysis can be obtained from even a miniscule biological sample, which allows investigating agencies to match crime scene evidence with suspects. However, because forensics is a science largely rooted in probabilities, even a confirmed "match" does not supply concrete proof of guilt. As in both scenarios the victims of crime were minor girls. So, during the act of sexual offence bleeding from genitals of victim is natural. While analysis, the forensic expert probably expects either blood of victim or semen of accused on garments as well as on crime scene. But every time, it doesn't happen. Many times, accused tries for intercourse but being minor the vaginal opening is small and victim cries due to pain so, accused himself gets penile injuries in trying forceful intercourse, his blood may found on crime scene and garments of victim and accused. In both the cases analyzed in our laboratory, we got male DNA profiles from blood detected on clothes. When we inquired injury report of accused, it showed penile injuries while attempting the rape. In absence of semen on clothes or medical samples of victim; the DNA profile obtained from blood of accused itself proved that the profiles belong to accused, in addition to this, medical injury reports also support that both culprits got penile injuries. It is definitely a sufficient evidence to prove the guilt of accused in the court.

In addition, DNA databases designed to simplify the process of connecting past offenders to recent crimes are fraught with concerns involving individual genetic rights, as well as problems related to delayed sample entry, both of which hinder the ultimate usefulness of these databases. As a result, even though forensics is undeniably important to the modern justice system, its personal ramifications and ethical questions are topics of continuing discussion within the scientific, law enforcement, and legal communities.

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