

DNA Y-STR Typing is an Important Tool to Solve Criminal Cases

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Abstract

The POCSO (Protection of Children Sexual Offence), 376 (sexual assault), 302 (murder) cases are on a rise in India and so are the cases where men are falsely implicated for the same. Many cases go unreported owing to the social stigma attached. There have been cases where women misused these laws for personal gains. Mixtures of body fluids and/or tissues from a male and female are frequently encountered with sexual assault casework and other violent crimes. These mixed samples often contain trace quantities of male DNA present in a large background of female DNA. Typical samples can include vaginal swabs containing a small number of sperm cells, body swabs from female victims of oral assaults, and fingernail scrapings/clippings from female victims of violent assaults. Standard autosomal STR analysis may be unable to detect the minor male contributor in these types of samples, while the 17 Y-STR loci techniques can routinely detect a minor male contributor present at less than 1:1000. In POCSO sexual assault cases the minor victims becomes a wide range of target by accused. In such cases rape cum murder also becomes a new fashion of psycho mind accused to destroy the evidence. We present a similar case study where the crime showed signs of violence.

The male-specificity makes the Y chromosome especially useful in cases of male/female cell admixture, namely in sexual assault cases. On the other hand, the haploidy and patrilineal inheritance complicates the interpretation of a Y-STR match, because male relatives share for several generations an identical Y-STR profile.

The presence of Y-peak on Amelogenin locus in Identifier STRs in vaginal swabs of victim raised doubt of case being positive but Y-Filer STR helped in distinguishing the male contributor from the alleged accused. This conclusion strongly indicates the power of Y-STRs in forensic DNA analysis – it not only helps in identification of perpetrator.

The Y-filer technique is a powerful tool for the analysis of sexual assault cases that contain a suspect(s). A significant proportion of DNA cases contain a suspect who has been identified by conventional law enforcement techniques. For these

cases, the Y-filer technique is a powerful tool that can be used to include or exclude a suspect(s) associated with an investigation.

Key words - Forensic science; Rape cum Murder case; Y-STR; DNA typing

I. INTRODUCTION

According to the section 376, POCSO-2012 and 302 of the Indian Penal Code (IPC), “Whoever, except in the cases provided for in sub-section, commits rape with murder, shall be punished with hanging till death or imprisonment for life” [1]. In the 1980s, forensic biologists began to establish DNA as a pillar of the investigative process. There has since been prolific growth in the application of DNA to forensic cases, and today DNA is one of the most highly regarded tools available to the forensic scientists [2]. Autosomal STR analysis may not be possible if the sample contains an admixture of body fluids other than semen, such as in saliva/saliva mixtures, or fingernail scrapings comprising cells from the victim and cells from the perpetrator [2]-[3].

Sexual assault is a crime that disproportionately affects adolescent and young adult women [4]. It is because autosomal STRs allow the detection of minor components only if they account for more than 5% of the mixture, as the rule of thumb [4, 5]. Sex-typing based on amelogenin is an integral part of most identifier PCR multiplex kits widely used for gender determination and plays an important role in forensic casework and creating DNA database [6]. The unique biology of the Y chromosome has led to the extensive use in forensic studies in determining identity of male individuals and patrilineal relationships [7-13]. Haplotyping of the human Y chromosome by use of STR markers or Y-STR haplotyping is a method used to detect and differentiate male DNA. The methodology was developed in parallel with the autosomal STR analysis for human identification purposes and evaluated in a very similar way for forensic analysis. Shortly after the characterization and evaluation of the first Y-chromosomal STR polymorphism its usefulness in crime casework was demonstrated when a mixed stain from a vaginal swab of a raped and murdered

female victim was resolved by Y-STR analysis and a falsely convicted male was excluded [1]-[15]. Unambiguous detection of the male component in DNA mixtures with a high female background is still the main field of application of forensic Y-STR haplotyping. Y-STRs in combination with autosomal STRs will thus be employed preferentially in sexual assault cases [16]. The Y-STR haplotyping method has been validated and standardized between labs and is now widely used in forensic applications [17]-[19].

The intent is not for Y-STR loci to replace any of the core autosomal loci but to provide separate tool contributing otherwise unavailable information in a number of scenarios:

- For mixed stains where the proportion of female DNA is higher than the male DNA present (which is frequently observed in vaginal swabs collected after sexual intercourse)
- For cases of alleged sexual assault where tests for seminal fluid or sperm are negative
- For sexual assault cases where the evidence in question is positive for semen, but no DNA foreign to the victim can be detected, or potential male alleles are below the threshold for autosomal STR detection
- For sexual assault cases where the evidence in question is amylase-positive and a male/female mixture is expected (e.g., traces of kisses or bites)
- For cases with very old semen stains, where the majority of sperm cells are suspected to be degraded and differential lysis is unsuccessful or risky
- For sexual assault cases where a large number of semen or other stains have to be screened
- For cases where the evidence in question is expected to include cells of a male perpetrator (e.g., female fingernail hyponychium where male biological material may accumulate after violent attacks)
- For cases where the patrilineal relationship of a stain donor needs to be determined
- For cases where the stain donor’s population of origin needs to be inferred [20].

Recently we investigated a sexual assault cum murder case reported by the Maharashtra Police team where they found a dead naked baby girl (aged 2 years) and the accused (aged about 36 years) at the scene of crime (accused home). According to FIR (first information report) “She was missing from her home and area where she played, there was no one other to see her. As she was kidnapped by accused and raped her forcefully. When she protested, shouted he started slapping and punching her. He also threatened to murder her if she shouted. He removed her cloths and raped her cruelly. Due to the afraid of police he killed her by strangulation. As he confirmed that she dead he hidden her at home and run away from the place. The police team tried to find her and searched suspected

homes for her but there was no clue about him. Finally police has come to known about the home which was locked from a week. Then finally they found the home place where her body was hidden by the accused .The police team was caught and arrested the accused”.

After medical examination of body, samples like superficial vaginal swab, deep vaginal swab, deep vaginal smear slide, superficial smear slide, anal swab, skin scrapping on thigh and abdomen of deceased and control blood sample of accused collected and sent M.O.(Medical Officer). Cloth article (i.e. full jean pant) forwarded by I.O. (Investigation Officer).

II. MATERIAL AND METHODS

The exhibits from hospital and I.O. i.e. superficial vaginal swab, deep vaginal swab, deep vaginal smear slide, superficial smear slide, anal swab, skin scrapping on thigh , abdomen of deceased and control blood sample, cloth article (full jeans pant) of accused were deposited at Directorate of Forensic Science Laboratory (DFSL), Mumbai by the police team for further processing. All the exhibits were subjected to DNA examination. DNA was isolated and preserved at 4°C. Dissolved DNA was used for DNA profiling.

A. Materials

Table 1: Chemical & Reagents:

Reagents	Parameters
Forensic Buffer	1 ml Tris HCL-100ml 0.5ml EDTA Buffer -10ml 5M Nacl-10ml Make the volume up to 1000ml
Proteinase K	Appearance- Colourless solution in 50% glycerol, cont.20mM Tris.,1mM CaCl ₂ ,PH ca.7.4 Concentration 20mg solid/ml
Investigat or kit	Buffer G2,Prot.K,Carrier RNA,
AmpFIST R® Yfiler® PCR Amplifica tion Kit	Allelic Ladder,AmpliTaq Gold®DNA polymerase, Primers
Primers	BDYS456,BDYS389I,BDYS390,BDYS389II,GDYS458,GDYS19, GDYS385,YDYS393,YDYS391,YDYS439,YDYS635, YDYS392,RYGATAH4,RDYS437

Hi-Di™ Formamide	CAS 75-12-7, CAS 60-00-4
Size Standard	GeneScan™-500, LIZ™

Instruments:

Table-2 EZ1 Automate DNA Extraction System Parameters

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
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 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	5.0°C/Sec.
Sample ramp rate	4.4°C/S
Temperature range	4-99°C/S
Temperature accuracy	±0.2°C
Customized programming	Allows a maximum of 20 steps and 99 cycles

Display	LCD touch screen, about 8.5 in
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Table-4 Genetic Analyser-3130

Instrument	Operating Parameters
Fragment Size(bp)	500bp
No. of Markers	17
Polymer	POP4
Detector	CCD
Oven Temp	60°C
Column Size	36cm
Software	Gene Mapper® ID Software v3.2.1

B. Methods

1. Extraction of DNA from swabs and stains

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



Take approx 0.5mm 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 Proteinase 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



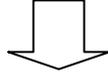
Set the micro-centrifuge sample tube in the EZ1 Advanced (Quiagen) magnetic bead based liquid handling system for automate DNA Isolation



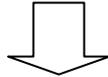
Store the extracted DNA at -20°C

2. Extraction of DNA from Blood

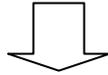
Take 5 µl blood sample+ 97.5 µl ATL buffer+100µl AL buffer+10 µl Proteinase K in micro-centrifuge Tube



Vortex and spin for 1 min's



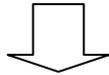
Incubate at 56°C for 10 min's on Thermo-shaker



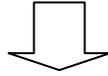
To this add 50 µl 99% ethanol then vortex and spin



Wait for 5 min's and transfer the supernatant into the micro-kit column



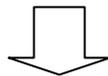
Centrifuge at 8000rpm for 1 min's and discard the filtrate



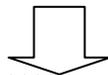
Wash the column by adding 500 µl washing solution



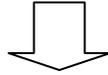
Centrifuge the column at 8000rpm for 2min's and discard the filtrate



Repeat the procedure



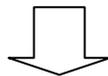
Spin the empty column at 10000rpm for 3min's



Transfer the column in new Elution Tube



Finally add 100 µl Elution Buffer to the column, centrifuge at 14000rpm for 2 min's



Remove the column and store the DNA for at -20°C

C. Polymerase chain reaction

1. Reagents Volume

STR genotyping was carried out using the AmpFISTR Y-filer PCR Amplification kit (Applied Biosystems, Foster City) shown in fig.1

AmpFISTR® Yfiler® PCR reaction mix:

10.5ul

AmpliTaq Gold DNA polymerase:

0.5ul

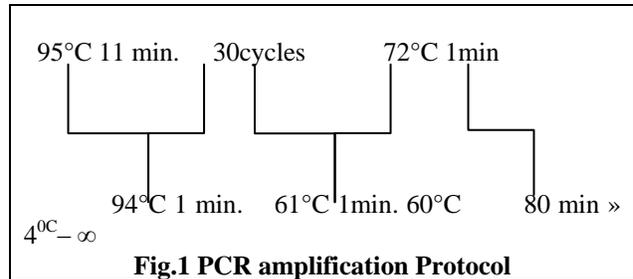
AmpFISTR® Yfiler® Primer set:

5.5ul

DNA Sample:

10ul

2. Protocol



D. Genotyping

STR genotyping is detected and analysed on 3130 Genetic Analyser (Applied Biosystems) instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.

1. Sample Preparation for Injection

Standard Mix:-

1µL Single or pooled PCR product

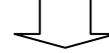
0.5 µL Size standard (for GeneScan500-LIZ®only 0.3 µL)

10-20 µL Hi-Di™ formamide (PN 4311320)

2. Protocol:-

Denaturation of PCR product

(90 -95 °C, 2 -5 min)



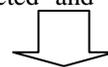
Immediately on ice or cool to 4 °C in thermal cycler Load the mixture in auto sampler on instrument for injection.



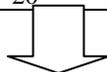
Electrophoresis is done through fine glass capillary filled with polymeric gel.(During capillary electrophoresis, the extension products of the PCR reaction (and any other negatively charged molecules such as salt or unincorporated primers and nucleotides)

enter the capillary as a result of electrokinetic injection. The extension products are separated by size based on their total charge)

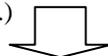
can be detected and distinguished in one capillary injection.)



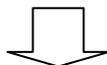
STR LOCUS	GENOTYPE						
	Exhibits collected by Medical Officer					Exhibits collected by Investigation Officer	
	Vaginal swab	Vaginal smear	Anal swab	Scrapping from thigh & abdomen	Control blood of Accused	Semen stain on full pant of Accused	Blood stain on full pant of Accused
BDYS456	15	15	15	15	15	15	15
BDYS389I	14	14	14	14	14	14	14
BDYS390	25	25	25	25	25	25	25
BDYS389II	33	33	33	33	33	33	33
GDYS458	17	17	17	17	17	17	17
GDYS19	15	15	15	15	15	15	15
GDYS385	11,15	11,15	11,15	11,15	11,15	11,15	11,15
YDYS393	13	13	13	13	13	13	13
YDYS391	11	11	11	11	11	11	11
YDYS439	10	10	10	10	10	10	10
YDYS635	23	23	23	23	23	23	23
YDYS392	11	11	11	11	11	11	11
RYGATAH4	12	12	12	12	12	12	12
RDYS437	14	14	14	14	14	14	14
RDYS438	11	11	11	11	11	11	11
RDYS448	20	20	20	20	20	20	20



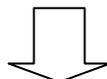
DNA fragments travel through capillary according to their size & reach the window which coincides with the Laser device in the instrument.(Shortly before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size, move across the path of a laser beam. The laser beam causes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



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

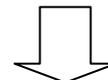


CCD Camera behind the window records the excitation peaks.(The dye signals are separated by a diffraction system, and a CCD camera detects the fluorescence.)



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,

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.)

IV. RESULTS AND DISCUSSION

The DNA extracted from,

1. Superficial vaginal swab, deep vaginal swab, superficial smear slide, deep vaginal smear slide, anal swab, scrapping on thigh and abdomen of deceased
 2. Blood stain and semen stain detected on full jean pant of accused
 3. Control blood sample of accused
- was typed at 17Y STR LOCI using Y-filer PCR amplification technique.

Table-5 Results of DNA typing are summarized below:-

V. INTERPRETATION

Table 5 represented the Y-STR profile of the exhibits collected,

a) DNA profile of male haplotypes obtained from Superficial vaginal swab, deep vaginal swab, superficial smear slide, deep vaginal smear slide, anal swab, scrapping on thigh and abdomen of deceased **matched** DNA profile of with the male haplotypes obtained from control blood sample of accused.

b) DNA profile of male haplotypes obtained from blood and semen detected on full jean pant of accused **matched** DNA profile of with the male haplotypes obtained from control blood sample of accused.

i.e. male haplotypes obtained from Superficial vaginal swab, deep vaginal swab, superficial smear slide, deep vaginal smear slide, anal swab, scrapping on thigh and abdomen of deceased and male haplotypes obtained from blood and semen detected on full jean pant of accused and male haplotypes obtained from control blood sample of accused are from the **same paternal progeny**.

DNA profiling can give a strong indication of guilt or innocence but the corroborative evidence may not always be supportive. Improper collection and preservation can weaken or destroy a potential source of facts in a case. The application of DNA profiling in the criminal investigations is an important aspect of criminal justice system today.

The forensic use of DNA Profiling is a major contribution to a technology which help in to reach the real culprit. The minor DNA component is generally undetectable below a ratio of 1:25–1:50 for autosomal mixtures. This diagnostic gap can be closed or at least reduced by Y-STR typing in an alleged sexual assault. Shortly after the characterization and evaluation of the first Y-chromosomal STR polymorphism its usefulness in crime casework was demonstrated by Roewer where a mixed stain from a vaginal swab of a raped and murdered female victim was resolved by Y-STR analysis and a falsely convicted male was excluded. In the present case, the first spark of doubt about the authenticity of the case flashed with the presence of accused DNA profile in any of the victim's exhibits. Moreover, the DNA profile generated from the Superficial vaginal swab, deep vaginal swab, superficial smear slide, deep vaginal smear slide, anal swab, scrapping on thigh and abdomen of deceased and blood stain, semen stain detected on full jean pant of accused was similar to the DNA profile generated from the blood sample of the alleged accused, which proved that the involvement of accused. Accused got punished for his 'violent behaviour'. In the present case, DNA fingerprinting technology was successfully applied in

solving the criminal case in our laboratory. The above illustrative case proved that DNA profiling is a tool that is used to apprehend the guilty. As it often helpful in the justice delivery system, conventional evidence.

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