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Y-STR: A Full Proof Technique for Male DNA Genotyping in Forensics

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Abstract

Crime statistics estimate that males are responsible for 80% of violent crimes and those males commit approximately 95% of sexual offenses. Sexual assault crimes account for a large percentage of biological evidence submitted to forensic casework laboratories. Identifying, segregating, and analyzing male DNA in sexual assault and other evidence containing mixtures of male and female DNA presents a complex challenge. Crime laboratories have traditionally employed a differential extraction method to separate spermatozoa from non-sperm cells. This technique is used to fractionate male and female DNA for analysis with autosomal typing methods. Due to the typically high quantities of female DNA encountered in sexual assault evidence, the ability to detect the minor male component may be limited. Detecting low levels of male DNA in a high background of female DNA samples, as is typically encountered in sexual assault cases is difficult. Y-STR chemistry is a powerful tool for the analysis of sexual assault cases that contain a suspect(s). The Y-Filer technique is the powerful tool & can be used to include or exclude a suspect(s) associated with an investigation.

Keywords: Forensics; Rape Assault; Y-STR; Genotyping

Introduction

The cause of this difficulty is that differential lysis of the male and female cellular fractions does not always result in complete separation of the fractions, resulting in difficulties in interpretation. Differential lysis is a laborious and time-consuming method of separation possibly when the male contributor has undergone a vasectomy or produces azoospermic semen. It is difficult to analyze body fluids from more than one individual. In such cases, preferential amplification of the major component of the mixture (usually female DNA) can mask the genetic profile of the male component [1]. It is because the autosomal STRs allow the detection of minor components only if they account for more than 5% of the mixture [2,3]. Sex-typing based on amelogenin is an integral part of most identifier PCR multiplex kits. Gender determination plays an important role in forensic casework and creating DNA database [4]. 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 [5-11]. 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 [12,13]. 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 [14]. Y-STR haplotyping method has been validated and standardized between labs and is now widely used in forensic applications [15-17].

A sexual assault case reported where a lady was brutally raped by four suspects in slum area. The accused were absconding from the scene of crime. According to FIR (first information report) "The lady (victim) was working as a maid and went to meet her friend in slum area. Victim's friend requests her to wait for an hour that she will return by work. at her home. During the period the suspects entered into the home and close the door. The victim cannot understand the situation and she became afraid by the persons. The suspects threatened to kill her and said to cooperate them. The four suspects raped her forcefully one by one and abscond from scene of crime. When her friend returned, they registered a FIR against unknown persons. After a month police team was caught and arrested the suspects.

It is very difficult to do the selection of semen stain of different accused from bed sheet. By different stain selection we segregate the stains from different location. DNA analysis which helped to connect the real accused in this case.

Vaginal swab of victim, control blood sample of accused, exhibit like bed sheet, condom collected from the scene of crime.

Materials and Methods

Chemical and 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, 20mM Tris.,1mM $Cacl_2$, PH ca.7.4, Concentration 20mg solid/ml
Investigator kit	: Buffer G2, Prot.K, Carrier RNA
AmpFlSTR® Yfiler® PCR Amplification Kit	: Allelic Ladder, Ampli Taq 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™

Detection of Blood and Semen on Crime Scene Exhibits

No blood detected on vaginal swab of victim and bed sheet; condom collected from scene was confirmed by Kastle-Meyer Test.

Semen was detected on vaginal swab of victim, bed sheet, condom collected from scene was confirmed by Acid-Phosphatase Test.

Extraction of DNA From Swab and Stains by Ez1 Auto Sampler Method

I. Carefully cleaned the platform of workstation of laminar flow with ethyl alcohol.
II. Approx 0.5mm sample piece and cut respective sample into small pieces and taken into 2ml micro-centrifuge sample tube.
III. Added 400µl Forensic Buffer +25 µl Proteinase K+40 µl 1mM DTT
IV. Vertex and spine.
V. Incubated at 56 °C overnight with Thermo-Shaker.
VI. To the next day, transferred the sample into the EZ1 micro-centrifuge sample tube.
VII. Set the micro-centrifuge sample tube in the EZ1 Advanced magnetic bead based liquid handling system for automate DNA Isolation.
VIII. Store the extracted DNA at -20 °C

Extraction of DNA from Blood by Micro Kit Method

I. 5 µl blood + 97.5 µl ATL buffer+100µl AL buffer+10 µl Proteinase K taken in to micro-centrifuge Tube
II. Vortex and spin for 1 min.
III. Incubated at 56 °C for 10 min's on Thermo-shaker.
IV. Added 50 µl 99% ethanol then vortex and spin.
V. Wait for 5 min's and transferred the supernatant into the micro-kit column.
VI. Centrifuge at 80000rpm for 1 min's and discarded the filtrate.
VII. Washed the column by adding 500 µl washing solution.
VIII. Centrifuge the column at 8000rpm for 2min's and discarded the filtrate.
IX. Repeated the procedure
X. Spin the empty column at 10000rpm for 3min.
XI. Transferred the column in new Elution Tube
XII. Finally added 100 µl Elution Buffer to the column, centrifuge at 14000rpm for 2 min's
XIII. Removed the column and store the DNA for at -20 °C

Polymerase Chain Reaction (PCR)

Reagents Volume: STR genotyping was carried out using the AmpFISTR Y-filer PCR Amplification kit.

I. AmpFlSTR® Yfiler® PCR reaction mix: 10.5µl
II. AmpliTaq Gold DNA polymerase: 0.5µl
III. AmpFlSTR® Yfiler® Primer set: 5.5µl
IV. DNA Sample: 10µl

PCR Protocol:

Parameter	Denaturation	Polymerase Activation	Annealing		Extension PCR (30) Cycles	
	Hold	Hold	Hold		Hold	
Temperature	95 °C	94 °C	61 °C	72 °C	60 °C	4 °C
Time	11:00 min	1:00 min	1:00 min	1:00 min	80:00 min	∞

Fragment Analysis: STR fragment analysis is done and analyzed on 3130 Genetic Analyzer instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.

Sample Preparation for Injection

Standard Mix:
Single or pooled PCR Product: 1µL
Size standard: 0.5 µL
Hi-Di™ formamide: 10-20 µL

Protocol:

I. Denatured of PCR product (90-95 °C, 2-5 min)
II. Immediately on ice or cool to 4 °C in thermal cycler
III. Loaded the mixture in auto sampler on instrument for injection.
IV. Electrophoresis is done through fine glass capillary filled with polymeric gel.
V. DNA fragments traveled through capillary according to their size & reach the window which coincides with the Laser device in the instrument.
VI. Laser excites the fluorescently labeled DNA fragments.
VII. CCD Camera behind the window recorded the excitation peaks.
VIII. Excitation peaks for 16 different loci are obtained.
IX. Run standard allelic ladder for each set of samples.
X. DATA Collected by software.

Results

It was very tricky job to extract the DNA from the samples collected by medical officer and scene of crime. There are always chances of cross contamination and mixing of foreign DNA. But we used specific reagents, chemicals and instrument to get the accurate results.

The table mentioned below shows that male DNA isolated from selected different stains on bed sheet, condom and vaginal swab matches with the DNA isolated from blood samples of four prime suspects in the crime. DNA was fragmented and run on the Genetic Analyser by using electrophoresis technique. The universal 17Y STR markers used in this method are listed in the table in first column. The unknown Electropherogram of male DNA derived from semen stains on bed sheet, condom and vaginal swab shown in column 2-7. Electropherogram of male DNA derived from control blood samples of suspects shown in column 8-11 was found matched. Table 1 STR typing are summarized below

STR LOCUS	GENOTYPE									
	Exhibits collected from scene of crime					M.O. Sample	Exhibits collected by Investigation			
	Bed Sheet				Condom	V. Swab from Victim	Control Blood samples of Accused			
	Stain 1	Stain 2	Stain 3	Stain 4			A1	A2	A3	A4
BDYS456	15	15	14	15	15	15	15	15	14	15
BDYS389I	13	12	13	13	14	12	13	12	13	13
BDYS390	24	22	24	23	25	22	24	22	24	23
BDYS389II	30	29	30	29	33	29	30	29	30	29
GDYS458	15	15	15	17	17	15	15	15	15	17
GDYS19	16	15	14	16	15	15	16	15	14	16
GDYS385	11,14	14,16	8,15	15,17	11,15	14,16	11,14	14,16	8,15	15,17

STR LOCUS	GENOTYPE									
	Exhibits collected from scene of crime					M.O. Sample	Exhibits collected by Investigation			
	Bed Sheet				Condom	V. Swab from Victim	Control Blood samples of Accused			
	Stain 1	Stain 2	Stain 3	Stain 4			A1	A2	A3	A4
YDYS393	13	11	13	12	13	11	13	11	13	12
YDYS391	11	10	10	10	11	10	11	10	10	10
YDYS439	10	12	11	11	10	12	10	12	11	11
YDYS635	23	22	20	21	23	22	23	22	20	21
YDYS392	11	14	11	11	11	14	11	14	11	11
RYGATAH4	12	12	11	11	12	12	12	12	11	11
RDYS437	14	15	14	14	14	15	14	15	14	14
RDYS438	11	10	10	9	11	10	11	10	10	9
RDYS448	20	15	18	19	20	20	20	20	18	19

*A: Accused; V.swab: Vaginal swab; STR: Short Tandem Repeats; M.O.: Medical Officer

Table 1: Represented the Y-STR profile of the exhibits collected **(A)** DNA profile of male haplotype obtained from vaginal swab of victim matched with the DNA profile of male haplotype obtained from control blood sample of one of the accused; **(B)** DNA profile of male haplotype obtained from condom collected from scene of crime matched with the DNA profile of male haplotype obtained from control blood sample of one of the accused; **(C)** DNA profile of male haplotype obtained from different semen stain detected on bed sheet collected from scene of crime matched with the DNA profile of male haplotype obtained from control blood sample of all of the accused

DNA profile of male haplotype obtained from vaginal swab of victim and bed sheet, condom collected from scene of crime and male haplotype obtained from control blood sample of all accused are found matched and from the same paternal progeny.

Discussion

The application of DNA profiling in the criminal investigations is an important aspect of criminal justice system today. 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 vaginal swab, 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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