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Research Paper

DNA Profiling for Detection of Mixed Semen Stain from Forensic Human Body Stain using Y-STR Analysis

Biotechnology

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ABSTRACT

In sexual–assault cases, DNA profiling of mixed stains is a challenge, as the presence of a large quantity of female material that may prevent detection of the male DNA. Here investigation was undertaken for DNA profiling for forensic mixed semen stain of cases of sexual assault. An efficient method is differential extraction for isolating sperm DNA from a mixed fluid sample which contains vaginal epithelial cells in a greater amount. The modified method of the two-step differential extraction procedure was found to be suitable for separating sperms DNA and vaginal cell DNA from the mixed stains. In this study the forensic sample which is having mixture of male and female DNA, Y profiles were obtained using Y-STR analysis.

DNA profiling or DNA fingerprinting is a molecular technique which describes the genotypes present at different locus along the chromosome. It has a variety of applications, ranging from aiding in criminal investigations to wildlife management. The process of DNA profiling, involves extraction of DNA from a specimen such as semen, blood or tissue and chemically divide the DNA into fragments. Because of the naturally occurring variations in the DNA molecule from one person to another, the sequence of fragments (STR) form a pattern similar to a bar code, that is to all intents and purposes unique to the individual (5). In sexual-assault cases, autosomal DNA analysis of gynecological mixed stains is a challenge as the presence of a large quantity of female material (Vaginal epithelial cells) may prevent detection of the male DNA (1, 9). A solution of this problem is differential extraction of DNA (4). These strategies are extremely sensitive and reproducible and confirm the reliability and discriminating power of DNA identification.

Materials and Methods

1. **Samples**: Cuttings of forensic mixed stain cloth from sexual assault case from the victim and four reference blood thread from victim and three accused.
2. **Y**-STR PCR ampFTA isolated DNA (ABI DNA protocol) (10).

DNA extraction from victim's cloth was done by differential extraction method (4) and DNA from control blood sample was done by FTA purification method. Quality and quantity of DNA from the mixed sample was check by gel electrophoresis. However for FTA purified DNA no quantification was required [DNA working procedure manual (RFSL-DNA-OPM)].

PCR Amplification: The master mix was prepared by combining PCR Reaction Mix, AmpliTaq Gold® DNA polymerase and AmpFℓY-STR® Yfiler™ Primer Reagents. Samples were prepared for PCR amplification and PCR (9700-ABI) was performed. DNA was amplified in a GeneAmp® PCR system for 30 cycles.

STR Analysis: Short tandem repeat (STRs) polymorphisms are variations in the human genome. STRs are short sequences of DNA about 2 to 5 base pairs that repeat many times adjacent to one another, typically within the non-coding region of DNA. STRs are examined by measuring the number of repeats of a certain STR sequence at a specific locus. Variability in STR regions can be used to distinguish one DNA profile from the other (2, 8).

First of all the cells were lysed to release the DNA and the DNA is precipitated by absolute alcohol then further purified by 70% alcohol and quantified. The DNA fragments produced by the PCR reaction are determined by running electrophoresis on 3130 DNA sequencer (ABI). The results of the analysis appear as peak on a computer output (Electophoregram) and these each peak correspond to a specific Y- Chromosome marker. Based on the location of the peak, the genotype software determined the exact number of repeats in the 16 DNA markers and thus obtained an exact haplotype (DNA profile) of that individual's DNA.

STR Data Analysis and Interpretation Software

Fragment analysis was accomplished by utilization of the 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the Westchester Forensic Lab procedure manual and GeneMapper®ID Software.

This is essential competition for the primers during PCR Y-STR amplification, which leads to preferential amplification of the major component of the mixture. In such cases, the use of Y-chromosome genetic markers, such as short tandem repeats (STRs), may only allow the amplification of even low quantities of male DNA independently of the victim's DNA.

Results and Discussion

It is generally accepted that the autosomal DNA characteristics cannot be detected by simple organic extraction and it is represented by two peaks of each Y-STR in electropherogram. So in mixed semen it is difficult to interpret the mixed STR (3). So it is done by differential extraction method by which male and female DNA is separated. Each fraction was then amplified by Y-STR amplification kit and run on DNA sequencer to get the Y-STR profile. Female DNA was not amplified, only male DNA was amplified.

DNA profiling of Y-STR was used to determine whether DNA characteristics of the male contributor could be retrieved.

Y-STR Analysis

Y-STR profiling from the DNA extracted from differential extraction (semen fraction) and simple organic extraction of reference blood was carried out. The alleles at sixteen Y-STR locus were determined. The Y-STR in all mixed semen profile shows the three to four allelic peaks. But in reference Y-STR profiling single locus have a single allele. While in female sample no peaks were observed as Y-STR is male specific. Table 1 show the peaks found at particular locus in semen of more than one individual. The presence of multiple peaks indicates that semen of more than one individual is present on victim's cloth. The presence of single peak on control blood sample indicates the Y-STR profile of each accused.

In table, the column -1 shows the 16-Y-STR locus that has been analyzed. The numbers in the table indicate the presence of specific allele on the particular locus. Column-2 shows the Y-STR profile of control DNA (007) provided by ABI. Column 3, 4, 5 shows the individual Y-STR profile of accused 1, 2 and 3. Column-6 shows the mixed Y-STR profiling found on the victim's sample. Victim herself has no Y-STR profiling so it is not represented in the table. The multiple peaks in the victims sample reveals that the presence of more than one semen samples.

All alleles of accused-1 that are 15,13,23,29,15,14,13,19,13,10,10,24,10,13,16,11,19 shown in column 3 are also present in the mixed Y-STR of semen fraction of victim's cloth (Column-6). Similarly Y-STR profile of accused-2 (Column-4) and that of accused -3 (Column-5) are present in the mixed Y-STR profile found on the victim's cloth (Column-6).

The above DNA evidence and typing procedures are uniquely useful in sexual assault cases because conventional analysis (ABO Blood grouping) cannot differentiate between similar blood groups found in secretion stains containing both seminal and vaginal fluids (6, 7). Therefore, if the rapist and victim had the same blood type, the scientist would not be able to determine from whom the sample was derived. DNA analysis eliminates this problem, as current technology can distinguish between the DNA from the victim and the offender. According to Y-STR profiling of forensic semen stain by differential extraction method to separate sperm fraction from the blood fraction from the victim and their comparison with the three reference Y-STR DNA profile, suggest that the multiple allelic peaks at single locus is due to the presence of mixed semen of all three reference blood sample collected from the three accused. The main advantage of the Y-STR approach is its ability to detect the three male components in the mixed stains however, the DNA from male contributors is present only in very small amounts.

So, by this study of DNA profiling of differential extracted DNA from mixed semen stain compared with directly extracted three reference blood DNA, it was found that all three reference semen DNA are present in mixed semen stain. So that study was helpful in sexual assault or rape cases investigation. Another benefit of the study is that minute amount of semen present in body stain like cloth from more than one male contributor can be identified using Y-STR analysis.

Table 1: Allelic peaks found on Y-STR profiling

Y-STR MARKER	CONTROL DNA	BLOOD REFERENCE -1 PEAKS (ACCUSED)	BLOOD REFERENCE -2 PEAKS (ACCUSED)	BLOOD REFERENCE -3 PEAKS (ACCUSED)	MIXED SEMEN FRACTION STAIN PEAKS (ON VICTIM'S CLOTH	VICTIMS BLOOD
B-DYS456	15	15	13	18	13,15,18	--
B-DYS 3891	13	13	15	13	13,15	--
B-DYS 390	24	23	24	25	23,24,25	-
B-DYS 38911	29	29	-	30	29,30	-
G-DYS 458	17	15	17	16	15,16,17	-
G-DYS 19	15	14	-	16	14,16	-
G-DYS 385	11, 14	13,19	12	11,14	11,12,13,14,19	-
Y-DYS 393	13	13	13	13	13	-
Y-DYS 391	11	10	11	11	10,11	-
Y-DYS 439	12	10	10	10	10	-
Y-DYS 635	24	24	23	23	23,24	-
Y-DYS 392	13	10	-	11	10,11	-
R-DYS-GATA H4	13	13	13	12	12,13	-
R-DYS 437	15	16	14	14	14,16	-
R-DYS 438	12	11	11	11	11	-
R-DYS 448	19	19	-	20	19,20	-

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