# Y-Chromosome DNA identification of aspermic male offender from vaginal swab of victim

**Dr. Sarabjeet Singh**, Department of DNA Fingerprint, Forensic Science laboratory, Govt. of National capital Territory of New Delhi, 110085, N.D. India.
**Dr. Gagandeep Kaur**, Department of DNA Fingerprint, Forensic Science laboratory, Govt. of National capital Territory of New Delhi, 110085, N.D. India.
**Dr. Rakesh K Gorea**, Faculty Member, College of Medicine, Salman Bin Abdul Aziz University, , Al Kharj, -11942, Saudi Arabia

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**Corresponding author**

**Dr. Sarabjeet Singh**
Department of DNA Fingerprint, Forensic Science laboratory, Govt. of National capital Territory of New Delhi, 110085, N.D. India.

Phone: +919650172699
Email: drsinghdna@gmail.com

**Abstract**

Identification of the offender from spermatozoa and blood is the most often sought biological evidence in the examination of rape victims. Forensic finding of the spermatozoa plays an important role to confirm the recent episode of sexual intercourse. Problems arise in cases of digital penetration and vasectomized males. Criminals are getting smarter now days, they are using condom to commit rapes or ejaculate away from the body of victim. In such cases no sperm cells are found and conventional STR testing yields only a female profile. This study demonstrates the presence of the male epithelial cells in the vaginal tract as available proof of coitus. In many circumstances the application of PCR-based DNA typing methods results in the failure to amplify the minor (e.g., male) component of DNA mixtures due to competition with alleles from the major (e.g., female) component. In this study DNA profile from the aspermic semen, vaginal swab and a control sample of a vasectomized male was obtained for all 16 Y-STR loci namely DYS392, DYS390, DYS385 a/b, DYS393, DYS3891, DYS38911, DYS391, DYS19, DYS439, DYS438, residing on the Y-chromosome and Amelogenin to help the investigating agencies to prove that a male was involved in the offence and further investigation was needed.

**Keywords**: Forensic Science, Short Tandem Repeat, Y-Chromosome, DNA profiling, rape cases.



## Introduction

Y-STR testing has played a critical role in sexual assault cases. In 1997, the U.S. Department of Justice estimated that 99% of the offenders of sexual assault incidents were male, thus the ability of forensic examiner to obtain male DNA profiles from sexual assault swabs. Evidence samples can play a critical role in prosecuting these offenders. In many sexual assault samples, such as vaginal swabs, the amount of female DNA overwhelms the quantity of male DNA present. Male DNA can appear in the epithelial fraction due to premature lysis of semen or male epithelial cells present in the ejaculate[1]. In these situations, the true genotype of the male suspect can be masked by the female victim's profile, making interpretation difficult. The interpretational problems can be compounded in the absence of reference sample(s), such as in non-suspect sexual assault casework. The absence of spermatozoa can also make analysis more challenging in some instances. Aspermic samples are

not even tested due to a potentially low ratio of male: female DNA. The present study utilized a Y-STR 16 STR to determine the efficacy of Y-chromosome STRs in profiling male DNA from non-suspect sexual assault samples lacking visually identifiable spermatozoa[2]. Y-STRs not only offer the potential to improve the chances of obtaining DNA profiles of male perpetrators of sexual assault, it could also be useful to analysis evidence in cold cases, where conventional DNA methods of the time had failed. In a case in 1998, Y-STRs were profiled from 25-year-old evidence collected from two rape cases in Japan, in response to a retrial request by a condemned criminal. The Y-STR alleles from the vaginal swabs analyzed were found to be identical to those of the accused, confirming the original.

## Materials and Methods

### Selection of Specimens

This study is done from a sample collected from cervico-vaginal area of the victim. A control sample was taken from the ejaculate of a vasectomized volunteer.

**Extraction and of DNA:** DNA was extracted from aliquots (200 ml.) of concentrated semen (Vasectomized semen sample) using phenol chloroform extraction method. The total human DNA was quantified by slot blot hybridization using a quanti blot TM kit by ADB system. Reference blood sample from the donor were not run since the semen sample collected were from donor.

**Amplification:** The Y-Filer amplification kit is used for identification of Vasectomized sample. Each group of sample for amplification included a positive control (2-5 mg of male DNA, ATCC = 4514 and negative control 2-5 mg. of female DNA, ATCC = 4551). The 9700 thermal cycler (ADB) system was used for each amplification reaction. The amplification condition were 95º C for 10 min. 30 cycles of 94º C for 30 Sec, 59º C for 1 min. 70º C for 1 min, 60º C for 60min & 40º C until the sample were removed from the thermal cycler.

### Analysis of amplified product on the 3100 genetic analyzer

One microlitre of the PCR product or control was added to 9.5 u/ml Hi-Di formamide containing 0.6 u/ml. gene scan – 500 (LIZ) size standards. The samples were denatured at 95º C for 3 min. using the 9700 thermal cycler. Denatured products were analyzed on the 3100 genetic analyzer using performance optimized polymer 4 (POP-4), filter-set, and an injection time of 5s. The run time was 26 min (the time necessary to consistently elute the 450 base pair size, standard peak I GS 500 ROX). A matrix file generated by using the matrix standard FAM, ROX and TAMRA were used. The allele fragments in a sample with reference to alleles in allelic ladder were used. The macro first provides allele designation to all allele in the allelic ladder based on their sizes. The size of each allele fragment in a sample is then compared with the size of designated alleles at each corresponding locus and the sample allele is labeled with the appropriate genotype.

## Results

A total number of allele were identified in 16 locus and allele frequency distribution of Y-STR loci from vasectomized person are described below and the results for the 16 Y-chromosome polymorphic STRs *loci* are shown in Table 1. Alleles were confirmed by Genotyping. There were 6 types of allele no.13 to18 present in (B_DYS456)15 , 4 types of allele no.12 to 15 present in (B_DYS389I), 6 types of allele no. 21 to 26 present in (B_DYS390), 6 types of allele no. 27 to 32 present in (B_DYS389II) respectively. It can be seen that the DNA profiles obtained for the male and female fractions from the vaginal Swab correspond to those from the suspect and victim, respectively. Allele size of all vasectomized semen sample and high level of Y-STR diversity was noted. More interesting haplotype result comes from locus (G_DYS385), locus (Y_DYS635) as well as locus (Y_DYS392) in which haplotype duplication observed. Means more than two alleles come together in Table.2

## Discussion

In vaginal swabs, amplification made it possible to detect the Y-chromosome STR, whereas absence of spermatozoa in semen sample taken from vasectomized male donor can be explained by a number of factors including penetration without ejaculation, an aspermic assailant, a non penile penetration, or a prolonged post-coital interval. In this regard, vaginal inflammation, salivary enzymes and anal bacteria accelerate the sperm cell lysis. The interval between intercourse and the sampling is usually only for 3 days.. This well known fact often leads the doctors of the forensic unit not to take swabs beyond three days, because sperms are found in vaginal swab up to 3 days confirmed by

**Table1**: Individual Y-STR allele size of aspermic male semen sample.
**(Value observed genotype in semen sample)**
Locus Samples included vaginal swab and Reference Sample

| **Locus** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
|---|---|---|---|---|---|---|---|---|---|---|
| B_DYS456 | 17 | 17 | 16 | 16 | 16 | 15 | 15 | 16 | 15 | 16 |
| B_DYS3891 | 12 | 13 | 13 | 12 | 15 | 12 | 13 | 15 | 13 | 12 |
| B_DYS390 | 23 | 24 | 21 | 23 | 24 | 25 | 21 | 25 | 22 | 23 |
| B_DYS38911 | 30 | 28 | 27 | 30 | 32 | 31 | 30 | 32 | 27 | 31 |

**Table 2**. DNA observed more than one copies of allele and confirmed by electropherogram.

| S.Nr | Y-STR Locus | Allele Observed | Repeat Unit | Genotype Range |
|---|---|---|---|---|
| 1 | B-DYS456 | 4 | 13-18 | 13-18 |
| 2 | B-DYS3891 | 5 | 12-15 | 10-15 |
| 3 | B-DYS390 | 4 | 21-26 | 18-27 |
| 4 | B-DYS38911 | 4 | 27-32 | 24-34 |

- Amplification of Y-STR loci provides critical information during analysis of male female mixture sample such as rape case[3]**.** Analysis of mixture sample from rape cases, typically involve differential extraction of sperm cell followed by evaluation of autosomal STR marker. They were two allele types in some haplotype due to the duplicated tandem repeat structure on this locus. The present study reveals that the duplication of Y-STR loci verify with Y-filer amplification kit.

electropherograms allele no[4]**.** It should be pointed out that in this case, the male DNA will be obtained from "epithelial fraction", which the one is obtained after mild protease digestion. Altogether, this suggests that vasectomized sexual assault swabs potentially contain enough DNA (0.5-2 ng) for profiling the male component using Y-chromosome specific loci. Neat ejaculates from vasectomized males were typed at DYS-19, 389 I & II, 390, 391, 392, and 393 using amplification kits on an ABI Prism®. However, the primers and amplification conditions in this study yielded a non-specific DYS391-related peaks with high ratios of non-Y

**Table 3**: Observed allele in female, vaginal swab and male profile

| S. Nr. | Locus | Female | Vaginal Swab | Male |
|---|---|---|---|---|
| 1 | D851179 | 11,16 | 11,14 | 14,16 |
| 2 | D21311 | 28,30,24 | 28,30,29 | 28,29 |
| 3 | D75820 | 8,10,12 | 8,11 | 8,11 |
| 4 | CSF190 | 11,12 | 11,12,14 | 12,14 |
| 5 | D351358 | 14,15,16 | 14,16 | 14,16 |
| 6 | THOI | 6,9 | 6,9,10 | 9,10 |
| 7 | D135317 | 11,13,12 | 11,13 | 11,13 |
| 8 | D165539 | 11,12,13 | 9,11,12 | 9,11 |
| 9 | D2S1338 | 18,20 | 18,20,23 | 18,23 |
| 10 | D19S433 | 12,14, | 12,14.2 | 14.14.2 |
| 11 | VWA | 15,17,18 | 15,17 | 15,18 |
| 12 | TPOX | 8,9,10 | 9,10 | 9,10 |
| 13 | D18S51 | 12,14,13 | 12,14 | 12,14 |
| 14 | D5S818 | 11,13,12 | 11,12,13 | 11,13 |
| 15 | FGA | 20,26 | 20,24,26 | 24,26 |
| 16 | AMEL | X | XY | XY |

(female) DNA. Preliminary results indicate that post-coital vaginal swabs (with Vasectomized male) can be readily typed with this Y-filer electropherograms no. and result of Y-specific method for establishing an upper limit to the concentration of Y-chromosome DNA is post-coital mixtures would be useful, but the confirmation of result in Aspermic ejaculate sample from vasectomized male are comparable with autosomal STR marker analysis, because autosomal or identifiler analysis reveal only the female DNA profile. The impetus for this study was the knowledge that male cells are present in the cervicovaginal only for 3 days after rape or sexual assault by Vasectomized donor. (Table: 3)

**Conclusion**

The result shows that Y-chromosome analysis provides evidence of the presence of male cells in alleged female victims of sexual assault even when no sperms are detected. This is useful for non-penile penetration as well. The method shows the feasibility of haplotype determination on swabs initially characterized as 'negative' and confirmed by electropherogram allele no. implying transfer of epithelial cells form male to female and vice versa. This study also shows a way to future confirmation. In rape case where the suspect is arrested soon after the crime by police officer as it is known that cells shed from a female during sexual intercourse can be retrieved from the penis of suspect within a 48 hrs of post coital period. The suspect penile swabs can be used as potential source of DNA evidence. The results obtained from this study will form concrete base for further trials.

**Conflict of interest**

None declared

**References**


1. Betz A, Bassler G, Dietl G, et al. DYS STR analysis with epithelial cells in a rape case. *Forensic Sci Int.* May 15 2001;118(2-3):126-130.
2. Sibille I, Duverneuil C, Lorin de la Grandmaison G, et al. Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa. *Forensic Sci Int.* Feb 18 2002;125(2-3):212-216.
3. Curran JM, Triggs CM, Buckleton J, et al. Interpreting DNA mixtures in structured populations. *J Forensic Sci.* Sep 1999;44(5):987-995.
4. Honda K, Roewer L, de Knijff P. Male DNA typing from 25-year-old vaginal swabs using Y chromosomal STR polymorphisms in a retrial request case. *J Forensic Sci.* Jul 1999;44(4):868-872.