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Use of Y - Chromosome in Sexual Assault by Y-Plex ^{tm}6 Amplification Kit and 310 Genetic Analyzer

Sarabjeet Singh*, Ruchira Chaudhary*
Department of Zoology,
Govt. M.V.M., Bhopal-462042, M.P., India

Abstract : Currently, post vasectomized azoospermic semen sample were analyzed for short tandem repeat (STR) on the Y- Chromosome by using 310 Genetic Analyzer. It has been observed a wide variation was attributed to the number of epithelial or white blood cells that are present in these azoospermic sample. DNA Profile of the vasectomized males was obtained for all six Y-STR Loci and by amplification kit. The method developed in this study demonstrate that Y-STR is a sensitive method for the identification of the presence of male epithelial cell in the post coital vagina

Key words : Forensic science, Y- Chromosome, Short Tandem repeat, Y-STR, Y-Plex^{tm}6

Introduction :

In this study of DNA, Y chromosome probes were used to detect specific regions of gene sequences of Y- Chromosome interphase nuclei of intact epithelial cells. Short Tandem Repeat (STR)* is a genetic marker which has gained popularity due to its high power of discrimination in human identification. STR loci are polymorphic loci found throughout all eukaryotic genome. They characteristically consist of tandem arrays of short repeated sequences of 2-6 base pair in length. Polymorphism occurs when the number of copies of the repeat sequences present at a given STR locus varies between individual chromosome. Y-STR In recent Years, Y-Chromosome STRs (Y-STR) have become important in forensic analysis because of ease of amplification of Male DNA from a mixed DNA sample, where one of the donor is a male and other a female. In case where multiple males or contributors are there, the no of donor can be estimated to haploid nature of Y-STRs Y-Plex ^{tm}6 310 Genetic Analyzer

Material and Method :

Collection of Sample : Semen samples form the six donor were collected in a sterile tube. After liquefaction an aliquot of semen was placed on a glass slide. The liquefied semen sample were centrifuged at 300rpm for 10 min. The supernatant (Homologous Seminal Plasma) and the cell pellet was re-suspended by using a vortex. Post -coital Vaginal swabs were collected at 24 hr., 48 hr., 72 hr, 96 hr and 168 hours, respectively, post coitus. Only one sample was obtained for each coital event. The one forensic sample that best mimics the situation would be taken after a sexual assault. Abstitence samples were obtained 3 weeks or more after the previous sexual contact. For swabbing the cottons swab was inserted

* **Corresponding author :** Sarabjeet Singh, Department of Zoology, Govt. M.V.M., Bhopal-462042, M.P., India Email : reachtojeet@yahoo.co.in

approx 1 inch into the vaginal canal , rotated for 5-10 min. and then carefully removed. Swabs were air dried for 1 to 2 hours at room temperature.

Buccal Swab sample were taken from both male and female volunteer for use as positive and negative controls. Y- Plex^{tm}6 Amplification Kit was used in each group of sample for amplification which included A Positive control:-(2-5 ng of male DNA ATCC # 45514). A Negative control:- (2-5 ng of Female DNA ATCC#45510) 5xy-Plex tm6 Primer was mixed with 0.5 ml reagent in 14.5ml of sterile water. 9700 thermal cycler was used for each amplification reaction at 95° C for 10 min, 94° C for 59 sec, 70° C for 1 min (30 cycle), 60° C for 1 Min, and 4° C for 60 min., until the sample were removed from the thermal cycler.After then the product is analysed on 310 Genetic Analyzer.

Microlitre PCR control is added :- 25.0 ml - HiDi Formamide 0.5 ml gene scan-500 (ROX) Size standard in 200 ml tube. Then samples were denatured at 950 C for 3 min using 9700 thermal cycler. Denatured Products were analyzed using Performance Optimized Polymer 4 (POP- 4), filter, set A, and Injection time of 5s. Run time was 26 min (the time necessary to consistently elute the 450 base pair size standard peak in GS 500 ROX) and A matrix file generated by using the matrix standard peak in GS 500 ROX .FAM, JOE, TAMRA was used.

Results & Discussion :

Amplification of Y- STR Loci provide critical information during the analysis of male female mixture sample such as those found in sexual assault case. Analysis of a mixture sample from a rape case. Typically involve differential extraction of sperm cell and female epithetical cell followed by evaluation of autosomal STR Loci. Many time, a complete separation of male and female sample is difficult to achieve and high amount of female DNA in mixture sample result in prefrential amplification of the female victim's DNA. This conventional approach has more limitation when the male donor in the rape case is either vasectomized or azoospermic (Butler & Devaney, 2001). In such case, the mixture sample gives a positives result for P 30 test (Gamma Semino protein).

Quantity of DNA isolated form 200 ml of semen

Sample	Yield of DNA (ng)
AZS-1	60
AZS-2	250
AZS-3	12.5
AZS-4	500
AZS-5	1000
AZA-6	120

The Random match probability by Y-Plex ^{tm}6, the Male profile is typed and single peak at each locus (Except for DYS385) enables once to determine the no. of male contributors.Eg. Multiple assault case.

A wide variation ranging from 12.5-1000 ng in the yield of extracted DNA was observed in all semen sample. The variation in yield is attributed to the varied no of epithelial / or WBC cell that were present in these semen sample. Epithelial and white blood cell were evident is microscopic evaluation of semen sample.

Cellular Y- Signal for each post coital sample, each day identification, of how many Y signal in epithelial cells were found from

Post-coital cell identification

Days after Coital Event	Y Signal Cell count / 125 cell
Days 0 non ejaculate	1, 3
Day 1	1, 6, 1, 6, 4
Day 2	3, 4, 4, 3 also O/29
Day 3	3, 1, 3, 4, 1
Day 4	2, 3, 3, 4, 2
Day 7	2, 4, 2, 1, 3
>= 21 days	0, 0, 0, 0, 0

each slide, for each day or instance counting a total 125 cells per slide.

Note - Each No. represent a separate post coital episode

Conclusion :

In this type, developing Y-Plex^{TM}6 amplification kit with various types of Y chromosome probes for epithelial cell identification and comparing the out come to current method from the same samples, gives excellent information regarding the use of this DNA based method for forensic purpose. No false positive in the Male or Female slide were observed. In each of the four male and four Female slide, there was correct identification of the Y Signal. The Y-Plex^{TM}6, Y average signal positive for the male cells

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