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TECHNICAL NOTE

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DNA Profiling of Azoospermic Semen Samples from Vasectomized Males by Using Y-PLEX™6 Amplification Kit

ABSTRACT: Post-vasectomized azoospermic semen samples (N = 6) were analyzed for short tandem repeats (STR) on the Y-chromosome by using Y-PLEX™6 and the 310 Genetic Analyzer. We have observed a wide variation in the yield of extracted DNA from 12.5–1000 ng. This variation was attributed to the number of epithelial and/or white blood cells that are present in these azoospermic samples. DNA profiles of these vasectomized males were obtained for all six Y-STR loci, namely DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385 amplified by using the Y-PLEX™6.

KEYWORDS: forensic science, Y-chromosome, short tandem repeats, DNA typing, human identification, polymerase chain reaction, Y-STR, forensic casework, Y-PLEX, DYS393, DYS19, DYS389II, DYS390, DYS391, DYS385

Short tandem repeat (STR) genetic markers have gained popularity due to their high power of discrimination in human identification (1). In recent years, the Y-chromosome STRs (Y-STRs) have become important in forensic analysis because of the ease of amplification of male DNA from a mixed DNA sample wherein one of the donors is a male and the other a female (2–4). In cases where multiple males are contributors, the number of donors can be estimated due to the haploid nature of Y-STRs. A number of Y-STR markers are now available to the academic and forensic community (3–5). Several of these markers are tetra nucleotide STRs and have been characterized extensively across several worldwide population groups for the allelic variants and their respective frequencies (6,7).

We have developed the Y-PLEX™6 multiplex system, which enables simultaneous amplification of six STR loci on the Y-chromosome, namely, DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385 (8). In the present study, we describe application of Y-PLEX™6 in the analysis of azoospermic semen samples from vasectomized men.

Materials and Methods

The Y-PLEX™6 amplification kit was from ReliaGene Technologies, Inc. (New Orleans, Louisiana). AmpliTaq Gold™ and reagents for the 310 Genetic Analyzer were obtained from Applied Biosystems (Foster City, CA).

Collection of Samples

Post-vasectomized semen samples from the six donors were collected in sterile tubes. The donors had undergone the surgery 3–4 months prior to collection of the semen. After liquefaction, an aliquot of the semen was placed on a clean glass slide and observed for the presence of any spermatozoa using a phase contrast microscope at 200 × magnification. The liquefied semen samples were centrifuged at 300 × g for 10 min. The supernatant (homologous seminal plasma) was decanted, leaving about 0.5 mL of the homologous seminal plasma. The cell pellet was re-suspended in the residual homologous seminal plasma by using a vortex. A small aliquot of this concentrated semen was again examined for the presence of any motile or non-motile spermatozoa. A sample was classified as azoospermic when spermatozoa were not observed in 60–80 high power fields (200 × magnification). Only azoospermic samples were used in the present study.

Extraction and Quantitation of DNA

DNA was extracted from aliquots (200 μL) of concentrated semen samples using a phenol-chloroform extraction procedure (9). The total human DNA was quantified by slot blot hybridization using a QuantiBlot™ kit (Applied Biosystems, Foster City, CA) following manufacturer's recommendations. Reference blood samples from the donors were not run since the semen samples collected were from single donors.

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Amplification

The Y-PLEX™ 6 amplification kit was used for these studies. Each group of samples for amplification included a positive control (2–5 ng of male DNA, ATCC #45514) and a negative control (2–5 ng of female DNA, ATCC #45510). The amplification reaction contained 5.0 μL of 5X Y-PLEX™ 6 Primer Mix, 0.5 μL of AmpliTaq Gold™ (5 u/μL), 14.5 μL of sterile water, and 5.0 μL of DNA (2–5 ng). The 9700 Thermal Cycler (Applied Biosystems, Foster City, CA) was used for each amplification reaction. The amplification conditions were: 95°C, 10 min; 30 cycles of 94°C, 30 sec; 59°C, 1 min and 70°C, 1 min; 60°C, 60 min; and 4°C until the samples were removed from the thermal cycler.

Analysis of Amplified Product on the 310 Genetic Analyzer

One microlitre of the PCR product or controls was added to 25.0 μL Hi-Di formamide containing 0.5 μL GeneScan® − 500 [ROX] Size Standard in a 200 μL tube. The samples were denatured at 95°C for 3 min using the 9700 thermal cycler. Denatured products were analyzed on the 310 Genetic Analyzer using performance optimized polymer 4 (POP-4), filter set A, and an injection time of 5 s. The run time was 26 min (the time necessary to consistently elute the 450 base pair size standard peak in GS500 ROX). A matrix file generated by using the matrix standards FAM, JOE, ROX, and TAMRA was used. The alleles were typed by using Y-Typer software (8). Y-Typer, a genotyping macro, types the allele fragments in a sample with reference to alleles in the allelic ladder. The macro first provides allele designation to all alleles in the allelic ladder based on their sizes. The size of each allele fragment in a sample is then compared with the sizes of designated alleles at each corresponding locus and the sample allele is labeled with the appropriate genotype.

Results and Discussion

Amplification of Y-STR loci can provide critical information during the analysis of male-female mixture samples such as those found in sexual assault cases (10). Analysis of a mixture sample from a rape case, typically involves differential extraction of sperm cells and female epithelial cells followed by evaluation of autosomal STR loci. Many times, a complete separation of male and female samples is difficult to achieve and the high amount of female DNA in mixture samples results in preferential amplification of the female victim's DNA (5). This conventional approach has more limitations when the male donor in the rape case is either vasectomized or azoospermic and sperm cells are not present. In such cases, the mixture sample gives positive results for the p30 test (gamma-seminoprotein) but sperm cells are not observed in microscopic evaluation. Obtaining a male profile for autosomal STR re-

TABLE 1—*Quantity of DNA isolated from 200 μL of semen.*

Sample	Yield of DNA (ng)
VM–1	60
VM–2	250
VM–3	12.5
VM–4	500
VM–5	1000
VM–6	120

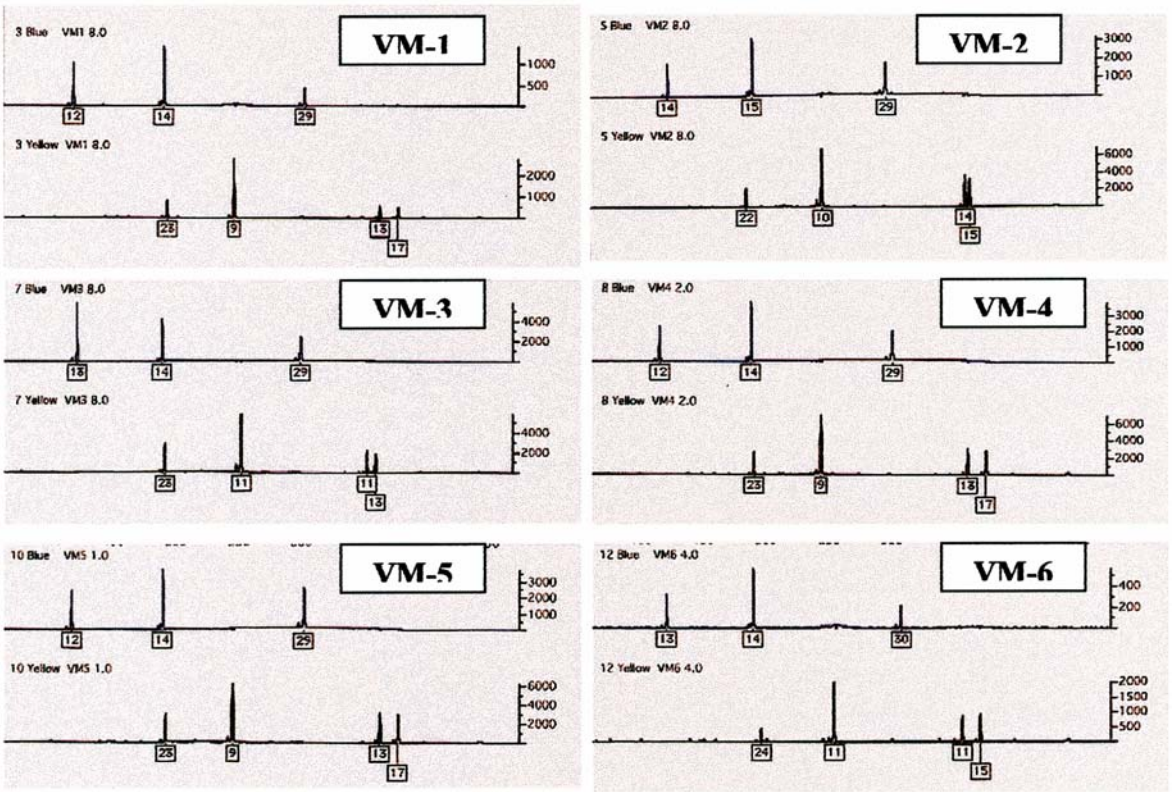

FIG. 1—*Y-STR profiles of the semen samples from vasectomized males (VM-1 to VM-6). The loci analyzed were DYS393, DYS19, and DYS389II (top portion of profile labeled as blue) and DYS390, DYS391, and DYS385 (bottom portion of profile labeled as yellow), sequentially.*

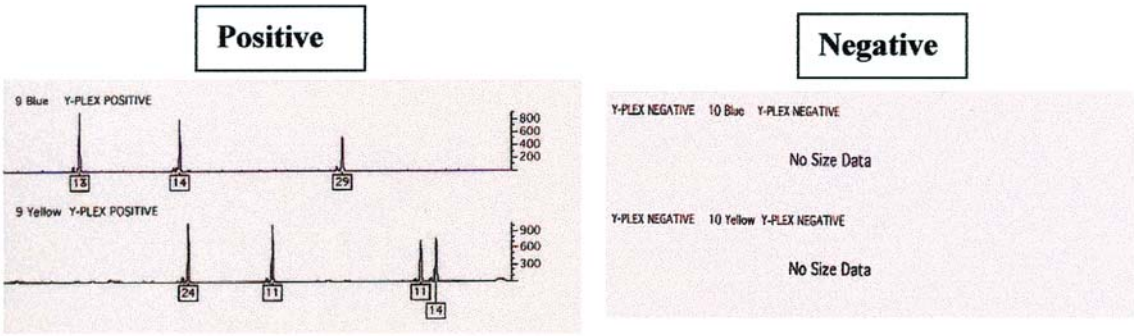

FIG. 2—*Profiles of the male positive and female negative controls amplified using the Y-PLEX™6. The loci analyzed were DYS393, DYS19, and DYS389II (top portion of profile labeled as blue) and DYS390, DYS391, and DYS385 (bottom portion of profile labeled as yellow), sequentially.*

quires enrichment of the male fraction, which is a tedious procedure. It is possible, though very rarely, to obtain a male contaminating profile in the mixture sample when sperm cells are absent; the male profile in such an event is due to the presence of male epithelial cells in the mixture sample. Use of amplification systems, which can specifically amplify Y-STR loci, should help in the analysis of mixture samples containing azoospermic semen. Use of the Y-PLEX™6 provides useful results in analysis of such samples.

The random match probability of the Y-PLEX™6 is 0.0096 and 0.0039 for Caucasian and African American population groups, respectively (8). Though the Y-PLEX™6 is not as distinctive as autosomal STR systems, it offers certain advantages in the analysis of mixture samples of male and female DNA; only the male profile is typed and a single peak at each locus (except for DYS385) enables one to determine the number of male contributors, e.g., multiple assault case. Autosomal STR profiles obtained in multiple assault cases often provide inconclusive results.

A wide variation, ranging from 12.5–1000 ng, in the yield of extracted DNA was observed in all six azoospermic semen samples (Table 1). The variation in yield is attributed to the varied number of epithelial and/or white blood cells that were present in these post-vasectomy concentrated semen samples. Epithelial and white blood cells were evident in the microscopic evaluation of the semen samples.

2.5 ng of extracted DNA from the vasectomized male samples was amplified and profiled using the Y-PLEX™6. Profiles were obtained for all six loci, namely DYS393, DYS19, DYS389II, DYS390, DYS391, and DYS385 for each of the six samples. Allele profiles are presented in Fig. 1. The results for male positive and female negative controls amplified with the samples are presented in Fig. 2. Two alleles were observed at DYS385 locus, which is a result of gene duplication and mutation and represents a characteristic of this locus (8,11). The lower rfu values for the loci in the blue window as compared to the loci in the yellow window for the sample VM-6 was probably a result of an amplification artifact. Correlation of the amount of DNA used for amplification and the extraction yield for the sample VM-3, suggest that one can obtain the DNA profile using as small as 40 μL of semen sample prepared from the vasectomized males.

Thus, DNA profile from azoospermic semen samples obtained from vasectomized males were obtained by using the Y-PLEX™6 amplification kit. This approach will be useful in the DNA analysis of rape case samples wherein the male contributor is vasectomized.

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