CASE REPORT

Use of PowerPlex® 16 and PowerPlex® Y Systems to Analyze a Complex Forensic DNA Mixture from an Incestuous Rape Case

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The PowerPlex® 16 System and later the PowerPlex® Y System were used to amplify and detect autosomal and Y-STR loci from a mixed DNA sample.

INTRODUCTION

DNA profiling has proved to be a powerful tool for forensic human identification. Nevertheless, analysis of mixtures presents certain challenges. Frequently, in sexual assault or rape cases, evidentiary samples contain material originating from more than one person. The detection and interpretation of mixtures, especially in incest rape cases, can be complicated by a number of factors such as loci with a less-than-four-allele pattern, low-quantity or degraded templates and many other technical issues regarding DNA analysis (1). Here we describe our experience with one case in November 2003.

A 15-year-old girl reported that her father had raped her. After the attack, she ran away from home and went to her mother's relatives. Police were contacted 24 hours later, and a suspect was arrested within 24 hours of police notification. Police collected the victim's clothing that she had worn for almost a whole day after the assault. During visual examination, police detected a weak stain on the victim's underwear. This stain had a weak positive reaction for acid phosphatase activity, indicating that the stain was biological evidence and could be seminal fluid containing sperm cells. Reference blood samples were taken from both the victim and suspect and were sent, together with other evidence, to the Laboratory for Forensic Genetics at the Institute for Genetic Engineering and Biotechnology in Sarajevo.

Application of Y-chromosome short tandem repeats (Y-STR) is preferable for analysis in these types of cases (2). However, because Y-STR kits were not available at that time, we used an autosomal STR multiplex system. Subsequently, we determined that autosomal STRs can be a highly informative tool in this kind of analysis.

MATERIALS AND METHODS

Reference blood samples were extracted using the Qiagen DNeasy®Tissue Kit. The seminal fluid stain was subjected to proteolytic digestion followed by organic extraction. This fragile trace was the only physical evidence that could prove contact between the suspect and victim, so we chose not to use differential lysis, which we had introduced but had not optimized as a routine procedure in our lab at that time. The PowerPlex®16 System[b–d], and later the PowerPlex®Y System[b], were used to amplify and detect autosomal and Y-STR loci, respectively. Numerical allele designations of the profiles were made using the PowerTyper™ 16 Macro. Interpretation of the mixture was performed according to the principles outlined by Gill *et al*. (3) and Evett *et al.* (4).

RESULTS AND DISCUSSION

The multiplex STR profile obtained from the stain suggested a mixed DNA profile with more than two peaks at seven loci (Figure 1). Because the number of alleles detected at each locus did not exceed three (with the exception of D21S11) we assumed that the mixture contained DNA from two close relatives (e.g., parent and child). Afterward, we compared the DNA profiles from both reference samples (victim and

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suspect) and noticed that D21S11 had an absence of shared alleles. The four-allele pattern allowed us to separate major and minor components, first visually and later by statistical analysis. The alleles in the minor component were specific for the suspect (father), while the major component matched the victim. We estimated the frequency of the minor component, deduced from the nonoverlapping alleles between the suspect and the victim (Table 1). It was approximately 3.49×10^{-10} in the Bosnian and Herzegovinian population (5).

Fortunately, we clearly observed a minor-component allele at all 15 loci. One step mutation detected on the D21S11 locus was beneficial because it generated a four-allele pattern, which is desirable in interpreting STR mixtures using allele peak areas. Earlier studies identified the D21S11 locus as one of the five STR loci with the highest mutation rate (0.21%, 6).

Approximately ten months later, twelve Y-chromosomal STR loci were used in re-examination of the same samples (suspect's reference sample and semen stain), and the results were confirmed with complete matching of the Y-STR profiles.

Table 1. STR Typing Results of the Forensic Evidence (Sperm Stain) and the Reference Samples from the Victim and Suspect*.

Locus	Victim	Semen Stain	Suspect
D3S1358	15, 19	**15**, **17**, 19	15, 17
TH01	9, 9.3	**9**, **9.3**	9, 9.3
D21S11	31, 32.2	**29**, **30**, 31, 32.2	29, 30
D18S51	12, 16	**12**, 16**, 18**	12, 18
Penta E	11, 14	11, **14**, **17**	14, 17
D5S818	11, 12	**11**, 12	11, 11
D13S317	11, 11	**11**, **13**	11, 13
D7S820	11, 12	**9**, **11**, 12	9, 11
D16S539	11, 11	**10**, **11**	10, 11
CSF1P0	12, 13	**11**, 12, **13**	11, 13
Penta D	11, 12	**11**, **12**	11, 12
VWA	17, 18	**15**, **17**, 18	15, 17
D8S1179	10, 13	**10**, **13**	10, 13
TPOX	8, 8	**8**, **8**	8, 8
FGA	21, 23	21, **23**	23, 23
Amelogenin	X, X	X, **Y**	X, Y

*Alleles given in bold could be assigned to the suspect upon re-analysis.

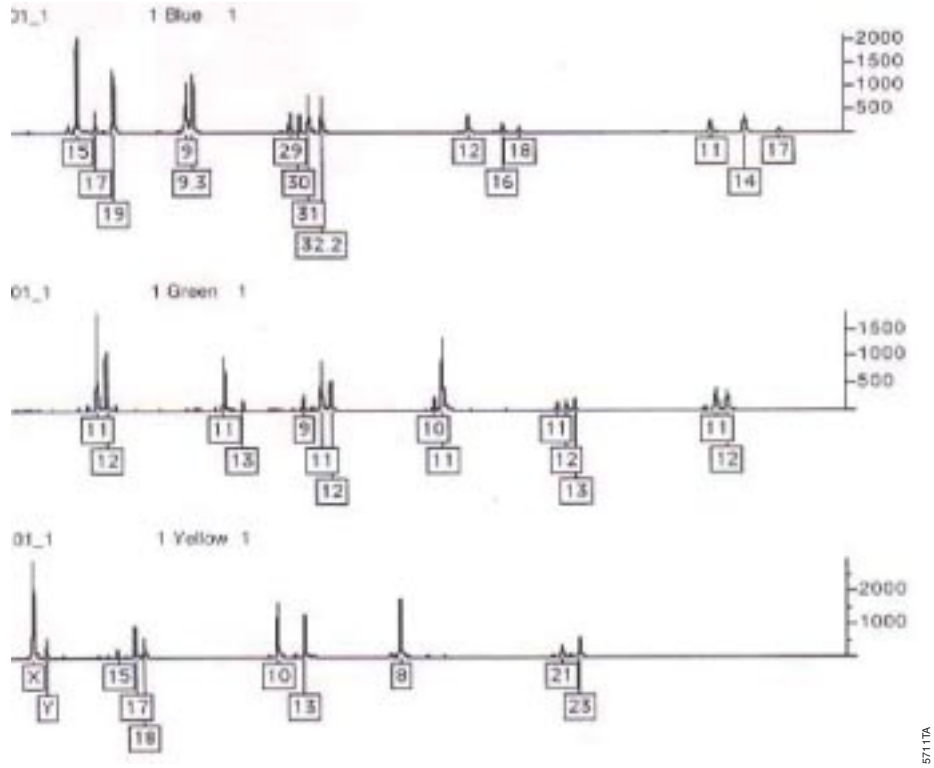

Figure 1. Electropherogram of amplified DNA from a semen stain showing a mixture with the major female (victim) component. DNA was isolated using organic extraction and amplified using the PowerPlex®16 System. The threshold was set to 70RFU. A four-allele pattern was detected only for D21S11.

REFERENCES

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