TECHNICAL NOTE

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Highly Informative Single-Stranded Conformation Polymorphism (SSCP) of Short Tandem Repeats in DNA Identification*

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ABSTRACT: The analysis of short tandem repeats (STR) by PCR is a useful technique widely used in DNA identification. Some loci have alleles differing in the number of complete repeat units, whereas others are polymorphic with alleles differing even to the level of a single base. Minor differences in the sequence of STR can confuse the analysis when the PCR product are poorly resolved in denaturing PAGE. The study by SSCP of STR in loci CSF1PO, TPOX, and THO1 and the alleles of locus D1S80 is shown in a representative case of paternity dispute. The analysis of short tandem repeats (STR) by the conformation polymorphisms of single-stranded DNA (SSCP) clearly enhanced the degree of resolution. The high resolution of SSCP of the STR may be the result of the arrangement of very prominent secondary structure and conformation due to the primary repetitive sequence. The method may be of particular interest to distinguish STR with similar PAGE mobility under denaturing conditions and to distinguish tandem repeats of the same size but different sequence.

KEYWORDS: forensic science, DNA typing, variable numbers of tandem repeats, conformational polymorphisms, disputed paternity, matching probability, nondenaturing PAGE, D1S80, CSF1PO, TPOX, THO1

The polymerase chain reaction (PCR) provides rapid and sensitive approaches for the analysis of highly polymorphic loci in human DNA. Short tandem repeats (STR) are a source of genetic markers widely used as a method for human identification (1). The amplification by PCR of polymorphic STR loci can be useful for identity testing in addition to restriction fragment length polymorphism (RFLP). Most STR loci consist of repeat units of 2 to 5 bp and allele sizes of about 100 to 300 bp (1,2). Highly discriminating multiplex STR can give matching probabilities in the range 10^{-8} to 10^{-10}, equal or greater than 4 to 6 single loci of variable nucleotide tandem repeat analyzed by RFLP (3). PCR products are separated by acrylamide gel electrophoresis and the degree of resolution depends on fragment sizes. Some loci have alleles differing in the number of complete repeat units, whereas others are polymorphic with alleles differing even by one bp. A set of loci are usually analyzed in DNA identifications to get a high combined probability. Nonetheless, alleles poorly resolved in a single locus may put in doubt the general interpretation. The rate of migration under nondenaturing conditions depends not only on the fragment size but also on the secondary DNA structure. This property has been successfully applied in the development of single-stranded conformation polymorphism (SSCP), a sensitive method for detecting sequence differences even to the level of a

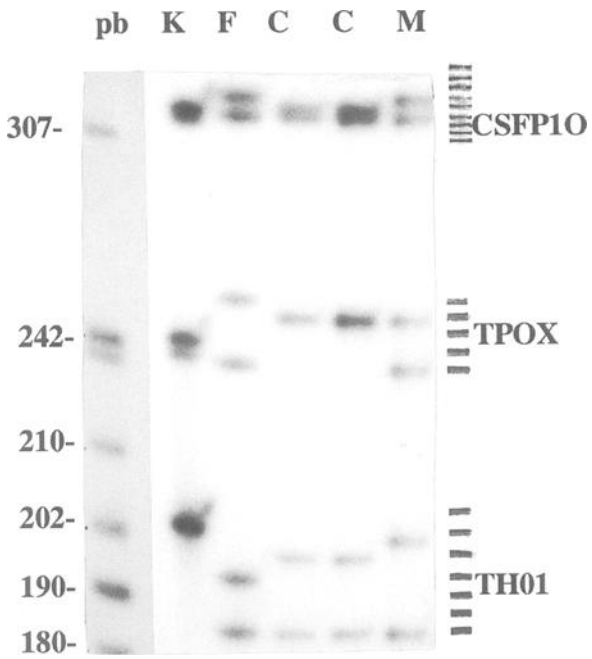

FIG. 1—*Analysis of loci CSF1PO, TPOX, and THO1 in denaturing acrylamide gels. Aliquots, 2.5 μL, of the PCR products of loci CSF1PO* (top), *TPOX* (middle), *and THO1* (bottom) *were mixed with 2.5 μL denaturing loading buffer (see Methods) and heated at 94°C for 2 min. PCR products were analyzed in 6% denaturing polyacrylamide gel electrophoresis containing 8 M urea at 55–60°C. Lane pb refers to bands of MspI-digested* ^{32}*P-labeled pBR322. K refers to K562 cells, F to alleged father, C to children (identical twins), and M to mother. Right handled lane shows hand-drawn marks of the migration of alleles 7–15 (CSFP1O locus), 8–12 (TPOX), 5–11 (THO1). The denomination of alleles refers to the number of repeats.*

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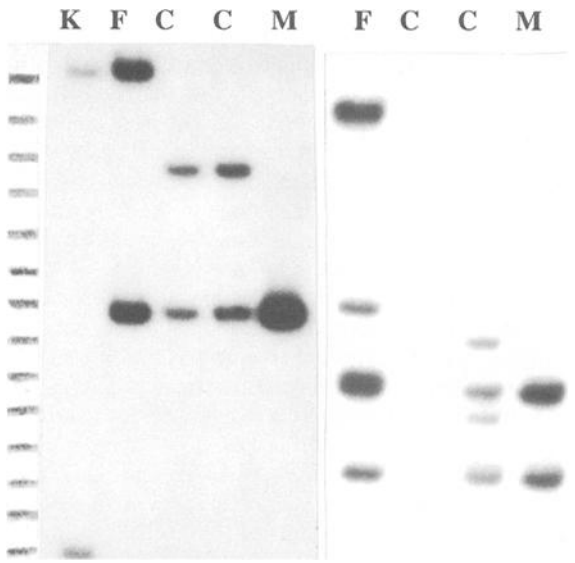

FIG. 2—*Double stranded DNA (ds DNA) and SSCP of locus D1S80. PCR products were mixed with denaturing loading buffer and heated at 94°C for 2 min and separated by denaturing (first four lanes left to right) or nondenaturing (last four lanes) PAGE. K refers to control DNA of D1S80 type 18/31, F to alleged father, C to children (identical twins), and M to mother. Left handled lane shows handdrawn marks of the migration of alleles 18 to 31.*

TABLE 1—*Haplotypes obtained from the alleles separated under denaturing conditions. Bands were identified in the autoradiogram and the alleles size calculated as described in Methods. AF refers to the alleged father.*

Locus	AF	Child-1	Child-2	Mother
CSF1P0	10/12	10/11	10/11	10/12
TPOX	8/12	11/11	11/11	8/11
TH01	8/5	9/5	9/5	10/5
D1S80	25/31	25/29	25/29	25/25

TABLE 2—*Haplotypes deduced from the bands of the SSCP. Bands were identified in the autoradiogram of SSCP and designated alphabetically from top to bottom. AF refers to the alleged father.*

Locus	AF	Child-1	Child-2	Mother
CSF1P0	ACCE	BCDE	BCDE	ACCE
TPOX	FHJK	GGII	GGII	GIJK
TH01	NRQS	MPQS	MPQS	LOQS
D1S80	TUXZ	VXYZ	VXYZ	XXZZ

single base (4). This is due to the effect of the conformation of DNA strands in the migration under conditions of nondenaturing gel electrophoresis.

In this paper, we demonstrate the practical advantage of SSCP to provide additional discrimination data from STR fragments of similar size in DNA identification.

Material and Methods

DNA was extracted from peripheral blood leukocytes by proteinase K digestion of the cells, salting out proteins and ethanol precipitation of DNA (5), DNA was quantified by the absorbance at 260

FIG. 3—*SSCP of loci CSF1PO, TPOX, and THO1. The products of multiplex PCR were mixed with denaturing loading buffer, heated at 94°C for 2 min and chilled in ice water. Electrophoresis was done in 6% nondenaturing acrylamide and the temperature of the plates was below 40°C. Bands can be identified in the loci CSF1PO* (top), *TPOX* (middle), *and THO1* (bottom). *K refers to K562 cells, F to alleged father, C to children, and M to the mother's DNA.*

nm. Alleles of loci LDLR, GYPA, HBGG, DTS8, and GC were detected by reverse dot blot with the PCR amplification and typing kit of Perkin Elmer. Approximately 40 ng of template, DNA were amplified in 100 μL of the PCR mixture. The STR of loci CSF1PO, TPOX, and THO1 were amplified with the primers supplied by Promega (GenePrint STR multiplex) according to manufacturer's instructions. The alleles of locus D1S80 were detected with the Perkin Elmer kit (AmpliFLP D1S80). Amplification conditions

were according to manufacturer's instructions with minor modification of supplementing 2 nCi/μL [^{32}P]-dCTP to 25 μL of the reaction mixture containing 25 ng of template DNA. PCR aliquots were run on 6% acrylamide without (nondenaturing gels) or with 8 M urea (denaturing gels) under the conditions indicated in the figure legends. STR products of the PCR were detected by autoradiography. Loading buffers used for all experiments are as follows: 1) Denaturing loading solution consisted of 10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol; 2) Nondenaturing loading solution consisted of 40% saccharose, 0.05 bromophenol blue, 0.05% xylene cyanol. The size of the alleles was calculated from the bands of MspI-digested ^{32}P-labeled pBR322, from the STR allelic markers produced by the manufacturer (Promega) and from the established STR alleles of K562 DNA amplified by PCR with [^{32}P]-dCTP.

Results and Discussion

A case of paternity dispute consisting of the alleged father, mother, and children (identical twins) was referred to our laboratory. The alleles of loci LDLR, GYPA, HBGG, D7S8, and GC did not exclude the paternity of the alleged father (not shown). Then we studied the STR of CSF1PO, TPOX, and THO1 loci and the allele of D1S80 locus in the samples of DNA of the reported individuals. STR consist of repeat units of 2 to 5 bp and allele sizes of about 100 to 300 bp. Because of the repetitive sequence, a very prominent conformation structure of the single stranded DNA is expected under nondenaturing conditions. Therefore, the PCR products were separated by PAGE under two different conditions (see also Material and Methods) and the results were the following: i) samples denatured at 95°C and run in denaturing PAGE supported the exclusion of paternity because alleles of the alleged father did not match allele 9 in locus THO1, allele 11 in TPOX (Fig. 1 and Table 1) and allele 29 in locus D1S80 (Fig. 2 left handled lanes and Table 1). Band sharing in locus CSF1PO (Fig. 1) is not clear due to minor differences in the migration; ii) samples denatured at 95°C and run in nondenaturing PAGE resulted in fully informative SSCP with haplotypes resumed in Table 2. SSCP alleles were alphabetically designated from top to bottom according to the migration rate. These results excluded the paternity too because the bands of the alleged father did not match BD, GI, MP, and VY bands (see Fig. 3, right handled lanes of Fig. 2 and haplotypes in Table 2). These results show that SSCP may be of particular interest when allele matching at a given locus is difficult to be ascertained due to minor differences in the migration of bands under denaturing conditions.

This study also showed that the alleged father, children, and mother shared at least an allele in all the loci studied with the exception of TPOX. This consideration is illustrative of difficulties that could be encountered when the mother's DNA is not available. Allele matching in a paternity dispute analysis relies on the calculations of matching probability obtained by the combined probability of several loci. However, the validation of the bands in a given locus depends on the degree of resolution and the potential sharing with the mother's alleles. Consequently, a match between the alleged father and child's alleles in a given locus is not very informative in the calculation unless one of the following conditions is given: 1) mother's DNA is available and does not match the allele, 2) child' alleles are in homozygosis, and 3) father and child share both alleles in case of heterozygosis. For instance, in the case noted in this paper, the discrimination at locus TPOX, clearly enhanced by SSCP, could be crucial if mothers's DNA had not been available. A very important advantage of SSCP is to show possible alleles of the same size but different sequence, that can not be separated by denaturing PAGE.

Single stranded conformation polymorphism is a sensitive method for detecting sequence differences even to the level of a single base (4). This is due to the effect of the conformation of DNA strands in the migration under conditions of nondenaturing gel electrophoresis. SSCP may be of particular interest when similar-size fragments of double stranded DNA coalesce into a single band resulting in the appearance of homozygosity. The high resolution of STR SSCP fragments may be the result of the arrangement of very prominent secondary structure and conformation due to the repetitive sequence. The method described here can be very useful in the analysis of poorly resolved bands and to distinguish tandem repeats of the same size but different sequence.

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