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TECHNICAL NOTE

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Analysis of STR-PCR products with high-resolution denaturing PAGE

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Abstract The Pharmacia Multiphor II horizontal electrophoresis chamber is a widespread tool for analysis of PCR products in forensic casework. Up to date, however, there is no protocol for successfully running high-resolution denaturing PAGE (poly-acrylamide gel electrophoresis) on a horizontal electrophoresis chamber. We modified the electrophoresis conditions to make this possible.

Key words Short tandem repeats · DNA-polymorphisms · Denaturing PAGE · Horizontal electrophoresis

Introduction

Several STR (short tandem repeat) loci are already available for forensic PCR analysis (Kimpton et al. 1992; Nishimura and Murray 1992; Wiegand et al. 1993). The most common ways of distinguishing between STR alleles that differ in length are native or denaturing vertical PAGE and native horizontal PAGE followed by silver staining (Allen and Budowle 1989). However, there is no description of a method for running STR-PCR products on a horizontal electrophoresis chamber such as the commonly used Pharmacia Multiphor II under denaturing conditions. We have developed a protocol that makes this possible. As examples, we show the results for the STR systems VWA (Kimpton et al. 1992) and F13B (Nishimura and Murray 1992).

Materials and methods

DNA isolated from 300 µl blood for RFLP parentage testing was used for PCR amplification of STR loci. DNA was isolated by phenol/chloroform extraction and ethanol precipitation according to standard protocols. The DNA was dissolved in sterile water and 2 µl of a 1 ng/µl dilution was used for PCR amplification using published primer sequences (Kimpton et al. 1992; Nishimura and Murray 1992). PCR was performed in 25 µl using the GeneAmp PCR System 9600 thermal cycler (Perkin Elmer). Amplification conditions were as described in Table 1. Before application, probes were denatured by heating to 95° C for 5 min and subsequent chilling on ice for 5 min and 8 µl was used for electrophoretic separation. Gel composition and electrophoresis conditions are given in Table 2. Precipitation of urea in the gel can be avoided by routinely applying 1 × TBE buffer along both short sides of the gel using a pipette before starting the electrophoresis. This creates a buffer barrier which prevents precipitation of urea. Buffer application is terminated about 1 cm before the agarose plugs. After the run DNA is visualized by silver staining according to standard protocols (Allen and Budowle 1989). For documentation dry the gel at 60° C for 1 h, then place an overhead sheet on the surface of the gel to prevent it from cracking.

Table 1 Amplification conditions for VWA and F13B (DynaZyme II DNA Polymerase, Finnzymes Oy)

VWA:	2 min 94° C 30 cycles of 15 s 94° C / 30 s 50° C / 30 s 72° C 5 min 72° C
F13B:	2 min 94° C 30 cycles of 15 s 95° C / 30 s 60 °C / 30 s 72° C 5 min 72° C

Results and discussion

The procedure described here makes it possible to perform denaturing high-resolution PAGE on a horizontal electrophoresis chamber such as the Pharmacia Multiphor II. In this way the advantage of denaturing PAGE (high resolution) can be combined with the simplicity of the Multiphor II in use. Separation of the alleles is improved about 2½-fold (see Figs. 1, 2). With the electrophoresis conditions, described here, the double bands that are common in denaturing PAGE can be

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Table 2 Gel composition and electrophoresis conditions

Denaturing conditions

PCR System	F13B	VWA
PAG	5.5%	6%
Urea	8.3 M	8.3 M
TBE	1 ×	1 ×

Mix and heat gently, filter, degas by vacuum, add 90 µl of TEMED and 12 µl of 10% ammonium persulfate / 30 ml of solution

40% acrylamide stock: 19 g acrylamide 2 × (Serva, research grade) 1 g N,*N*-methylene bisacrylamide (Serva) / 50 ml solution

Dissolve and stir for 30 min with 3.5 g Amberlite IRN 150 L (Pharmacia Biotech), filter, store at 4° C in a dark bottle

Agarose plugs: 2 % agarose (Sigma A-6013 type I: low EEO) in 1 × TBE/bromophenol blue

Electrophoresis conditions: 500 V, 10–12 mA, 10 W, 25° C
Prerun 30 min
VWA: 4 h
F13B: 5 h

Samples are applied along the long side of the gel (separation distance about 12 cm)
The optimum separation time is about twice the time the bromophenol blue front needs to pass through

Native conditions[b]

VWA and F13B:

PAG	6%
CHES	0.028 M
Tris/Formiat	80 mM

Agarose plugs in 2 × TBE

Electrophoresis conditions: 1000 V, 40 mA, 15 W, 10° C about 3 h

Samples are applied along the short side of the gel (separation distance about 16 cm)
The optimum separation time is about the time the bromophenol blue front needs to pass through

avoided. For a comparison of denaturing versus native electrophoresis conditions see Table 2. Examples are shown for the STR systems VWA (Fig. 1) and F13B (Fig. 2).

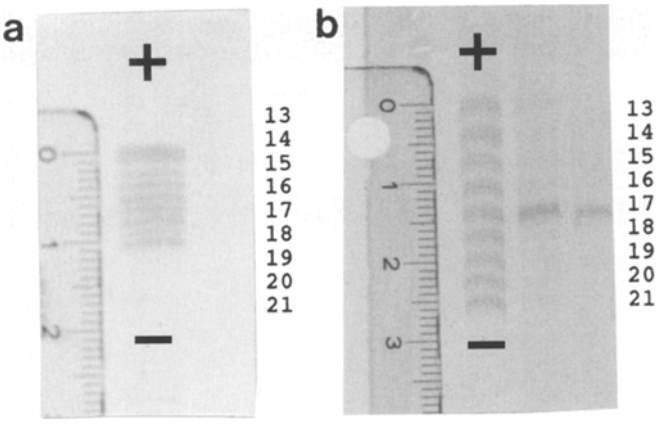

Fig. 1 VWA-PAGE according to **a** standard protocol (Wiegand et al. 1993), separation distance 16.5 cm, and **b** denaturing conditions on a horizontal chamber, separation distance 12 cm. Alleles 13–21 are shown; additional *lanes* show genotype 17/17

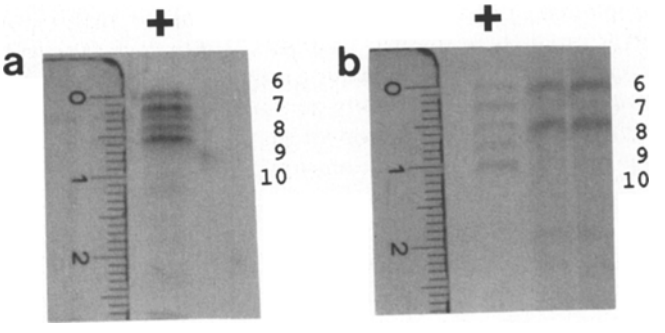

Fig. 2 F13B–PAGE according to **a** standard protocol (Wiegand et al. 1993), separation distance 15 cm, and **b** denaturing conditions on horizontal chamber, separation distance 12 cm. Alleles 6–10 are shown; additional *lanes* show genotype 6/8

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