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TECHNICAL NOTE

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High-resolution vertical PAGE: an alternative electrophoretic system with multiple forensic applications

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Abstract DNA profiling based on PCR technology has become a powerful tool in forensic casework, as it enables specific amplification of polymorphic VNTR loci from very small quantities or even degraded human DNA. Genetic typing of AmpFLP or STR loci may require electrophoretic separation techniques usually achieved by sequencing gels. In this report we present a non-denaturing high-resolution vertical PAGE system which can be easily used for both VNTR subgroups. The system has been evaluated for single- and multiplex use in routine casework and has been shown to be rapid, sensitivite and reproducible.

Key words VNTR polymorphisms · AmpFLP/STR analysis · Singleplex/multiplex · Electrophoretic resolution · Vertical PAGE

Introduction

The separation of PCR amplified VNTR alleles is commonly performed by horizontal discontinuous polyacrylamide gel electrophoresis (hd-PAGE), first described by Allen et al. (1989). In the past, several modifications on the PAGE technique have been used to improve electrophoretic separation. It has been reported that the usage of different trailing ions (Rand et al. 1992; Allen et al. 1993), pH alteration (Sajantila and Lukka 1993) and the use of stacking or composite gels (Lorente et al. 1993; Sajantila and Lukka 1993) or denaturing gels (Schumm et al. 1994) resulted in increased resolution. In the present report, we describe a more simple and reliable non-denaturing high-resolution vertical continuous PAGE-system (vc-PAGE) that, compared to previously described electrophoretic techniques (Budowle et al. 1991; Allen et al. 1993; Lorente et al. 1993; Sajantila and Lukka 1993), allows improved resolution over a wide range of PCR amplified VNTR alleles. Separation of alleles ranging between 100 and 1300 bp in length and differing in size by as little as 1 bp can be easily performed. Therefore, the system allows high-resolution typing of AmpFLP and STR systems without modifying the electrophoretic setup. Furthermore, based on its resolution capacity, one-tube multiplex analysis or sequential loading of multiple amplifications can be performed.

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Materials and methods

DNA samples

DNA was extracted from EDTA blood samples using the chelex extraction procedure (Walsh et al. 1991). Extraction of vaginal swabs containing semen was performed as described by Wiegand et al. (1992). The samples for the population study from Münster were taken from unrelated individuals involved in paternity cases, and PCR, electrophoretic separation and visualisation were carried out as previously reported (Wiegand et al. 1993).

PCR protocol

The AmpFLP and STR loci were amplified using primer sequences and reaction conditions as published previously for ApoB (Boerwinkle et al. 1989), D1S80 (Budowle et al. 1991), HUMTHO1 (Kimpton et al. 1993), HUMVWA (Kimpton et al. 1992), HUMFES (Polymeropoulos et al. 1991) and amelogenin (Sullivan et al. 1993).

Multiplex amplification conditions

Multiplex 1: HUMFES/HUMTHO1/amelogenin
94° C for 60 s, 60° C for 60 s, 72° C for 60 s, 30 cycles
Multiplex 2: HUMFES/HUMVWA/amelogenin
94° C for 60 s, 60° C for 60 s, 72° C for 60 s, 30 cycles

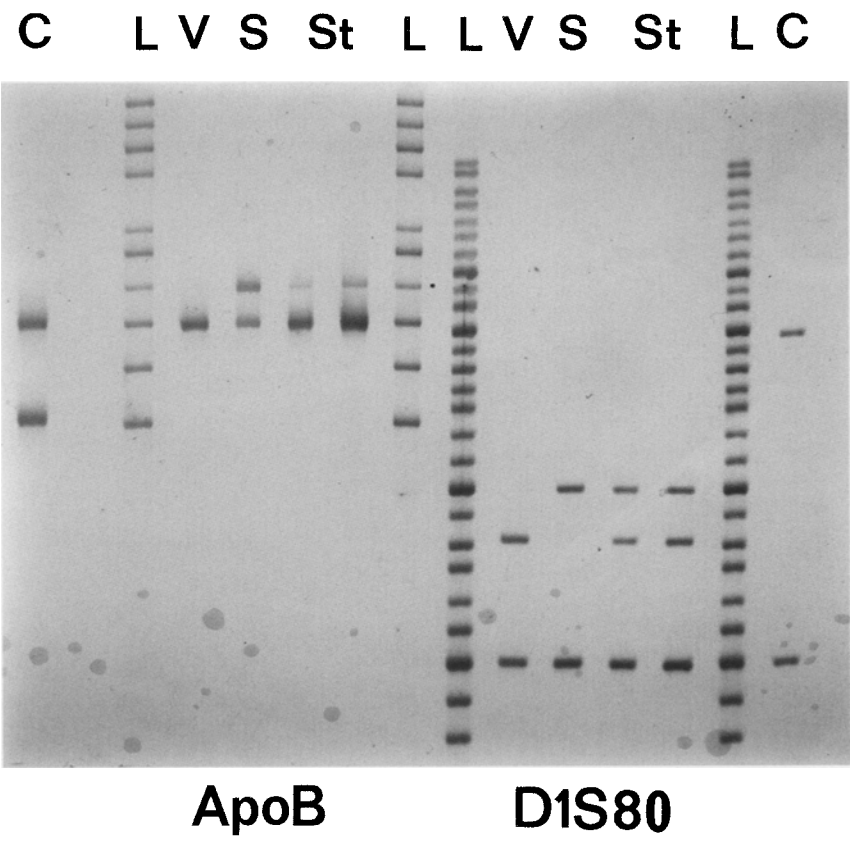

Fig. 1 Silver-stained vc-PAGE displaying ApoB and D1S80 profiles derived from a rape case and mixed stain analysis. Electrophoresis was conducted for 3 h at 750 V. *L* Allelic ladder, *V* victim, *S* suspect, *St* mixed stain achieved from a vaginal swab after mild preferenital lysis (Wiegand et al. 1992). For ApoB C (control) = 31/35 and for D1S80 C = 18/31. Anode is at the bottom. ApoB allelic ladder contains alleles 31–41 and 43–49, D1S80 allelic ladder contains alleles 16–41

Sample application and electrophoresis

Aliquots of 0.1–10 µl of amplified DNA were mixed with 2 µl of sample buffer containing 40% sucrose and 0.1% xylene-cyanol in a solution containing 200 mM Trizma Base, 100 mM Sodium acetate (anhydrous) and 1 mM EDTA in a total volume of 12 µl. For better sample loading, Multiflex FLAT tips (Sorensen, BioScience) should be used. Prior to sample loading it is essential to wash out the wells as soon as the comb is removed, otherwise small fragments of acrylamide can remain in the wells, producing irregularly shaped surfaces that will lead to diffuse or wavy bands. For multiple loading of the gels the xylene-cyanol dye was allowed to run 2.5 cm before loading the next set of samples. This prevents the samples from overlapping during electrophoresis. A 32 × 18 × 0.08 cm non-denaturing vc-PAGE was used to separate AmpFLP and STR alleles. Electrophoresis was carried out using the BRL SA32 sequencing apparatus at a constant voltage of 750 V using 1 × TBE as running and gel buffer. The gel temperature during electrophoresis should be maintained at 50°C to avoid "smiling" or even melting the DNA strands. The universal gel formulation used was 8% and 3.2%C (*N,N*-methylene bisacrylamide). Gels were cast between two unsealed glass plates. Casting the gels on GelBond (FMC) or glass plates treated with bind-silane is not advisable, as high background levels during silver staining and a loss of sensitivity can occur.

Allelic ladders

An allelic ladder consisting of a set of known alleles was analysed in parallel to the samples on each gel. The allele designation related to the number of repeats has been aligned to the nomenclature used in the present study (ISFH 1994).

Silver staining

Separated DNA bands were visualized by the silver staining procedure described by Budowle et al. (1991).

Results and discussion

Based on our previous experience with vc-PAGE AmpFLP analysis (Schneider 1993), it was our aim to adapt this flexible native electrophoresis system to the recently introduced STR/multiplex technology (Urquhart et al. 1994). To improve electrophoretic resolution only slight modifications have to be introduced. These include (a) extension

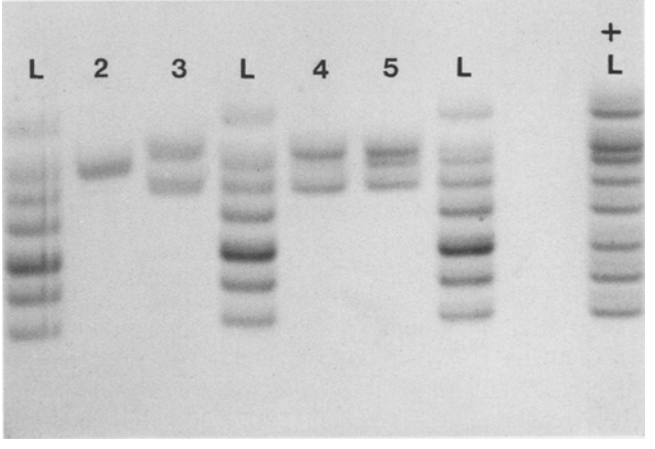

Fig. 2 HUMTH01 mixing experiment *(lanes 4, 5)* with samples containing alleles 9.3/9.3 *(lane 2)* and 9/10 *(lane 3)*. Electrophoresis was conducted for 3 h at 750 V. *L* Allelic ladder exhibiting alleles 5, 6, 7, 8, 9, 9.3, 11; *L+* Allelic ladder exhibiting alleles 5, 6, 7, 8, 9, 9.3, 10, 11

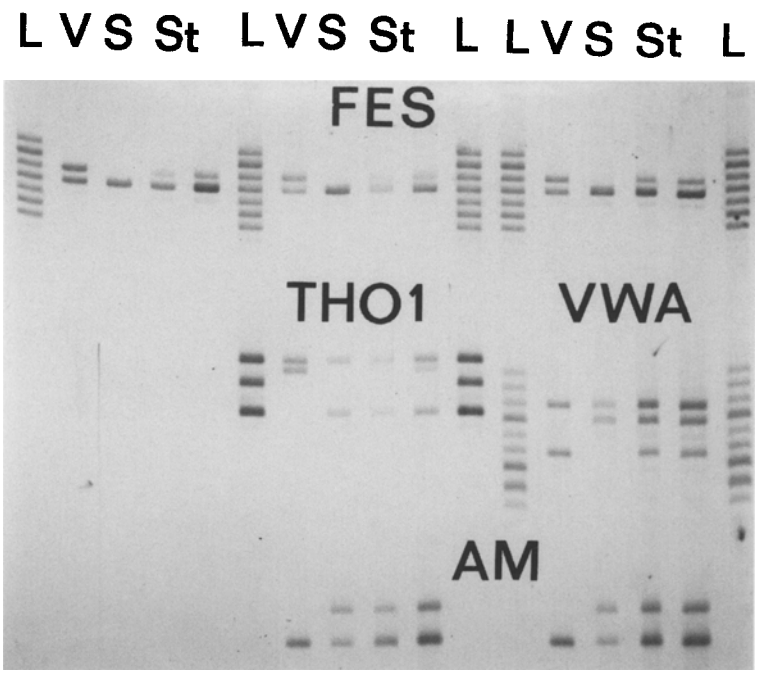

Fig. 3 Rape case amplification pattern after single- and multiplex STR analysis. Electrophoresis 2 h 30 min. *FES* Singleplex amplification with HUMFES/FPS *(left panel)*, *FES/TH01/AM* multiplex 1 with HUMFES/FPS, HUMTH01 and amelogenin *(middle panel)*, *FES/VWA/AM* multiplex 2 with HUMFES/FPS, HUMVWA and amelogenin *(right panel)*, *L* allelic ladder, *V* Victim, *S* Suspect, *St* mixed stain from a vaginal swab after mild preferential lysis. FES/FPS allelic ladder contains alleles 8–14, VWA allelic ladder contains alleles 13–21, and THO1 allelic ladder contains alleles 6, 8, and 9.3

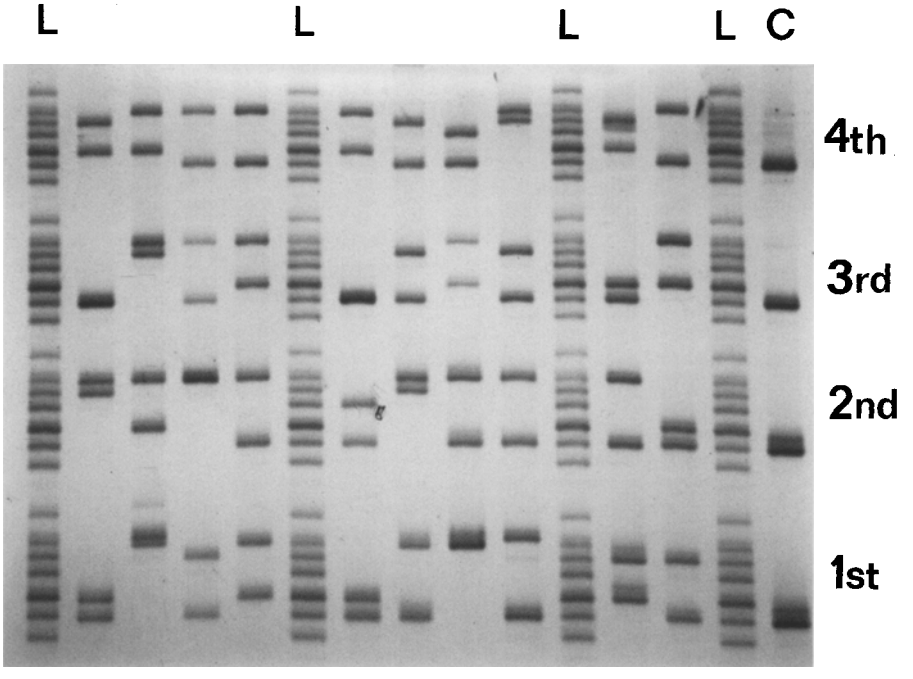

Fig. 4 Sequential loading of multiple casework samples amplified with HUMTH01. Samples and ladders were loaded at four different times: *1st* 0 min, *2nd* 20 min after load 1, *3rd* 40 min after load 1, *4th* 60 min after load 1. Electrophoresis was conducted for a total of 3 h 50 min at 750 V. *L* Allelic ladder containing alleles 5, 6, 7, 8, 9, 9.3, and 11. *C* Control

of the separation length from 20 to 30 cm, (b) increasing the acrylamide concentration to 8%, (c) lowering the gel thickness from 1 mm to 0.8 mm and (d) elevating the running temperature to 50° C. As shown in Fig. 1, these modifications resulted in sharply defined DNA fragments when analysing ApoB and D1S80 in parallel. Although allele sizes for the two loci vary from 300 to 1300 bp, both AmpFLPs can be analysed simultaneously. The most advantageous effect of the gel system, however, is that high-resolution STR analysis can be performed without changing the electrophoretic setup. To demonstrate the resolving capacity of our vc-PAGE system a mixing experiment with the HUMTHO1 alleles 9.3 and 10 was performed (Fig. 2) showing that this method is clearly capable fo resolving the 1-bp difference. The advantages of multiplexing PCR systems have been noted in several recent publications (Sullivan et al. 1993; Kimpton et al. 1993). Most notably, the multiplex approach offers both a high discriminating power and the advantage of saving sample material and analysis time. For routine casework, we usually use one of two triplex combinations which comprise the loci FES, TH01 and amelogenin (Fig. 3) or

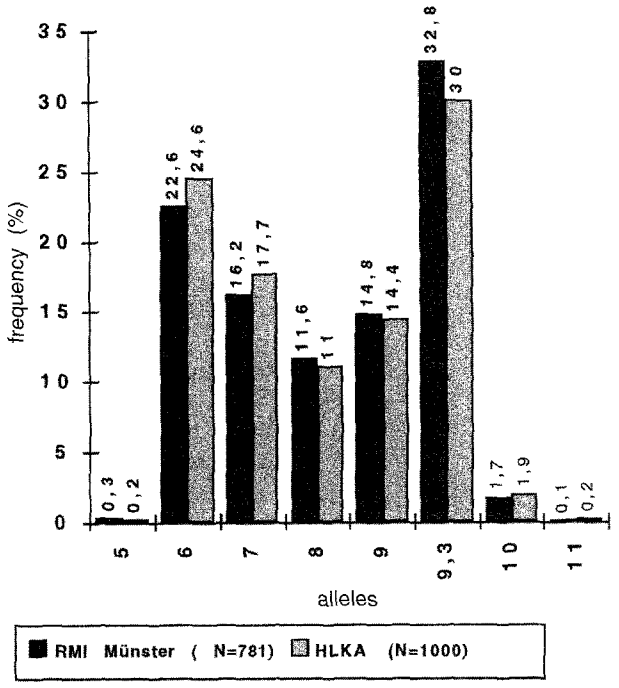

Fig. 5 Comparison of HUMTH01 allele frequencies obtained from two Caucasian populations and using two electrophoretic systems. *Münster* $n = 781$ individuals (hd-PAGE), *HLKA* $n = 1000$ individuals (vc-PAGE, multiple loading)

alternatively the loci FES, VWA and amelogenin (Fig. 3). Both STR-triplex combinations have proved to be highly robust and discriminating with the added advantage of direct gender identification. Although sex typing in the triplex reaction does not add greatly to the overall discrimination potential, it has been reported that with this system it is possible to detect a male signal in a 100:1 female to male mixture (Mannucci et al. 1994). Comparable multiplex systems have been reported previously for denaturing electrophoresis followed by manual silver staining (Schumm et al. 1994) or automated fluorescence detection (Kimpton et al. 1993; Sullivan et al. 1993). But with respect to the high-resolution capacity of our non-denaturing vc-PAGE, the present report describes a simple and time-saving multiplex approach that will possibly facilitate "low-cost" STR technology transfer between forensic laboratories. Besides its suitability for single- or multiplex VNTR analysis, the gel system further enables multiple time loading (Schumm et al. 1994), using samples from either the same or different loci. As shown in Fig. 4 for HUMTHO1, up to 50 samples can be easily analysed in less than 4 h. A qualitative comparison between our population data obtained from this multiple loading approach (HLKA) and data obtained from the Münster area (Fig. 5) using the method of Wiegand et al. (1993) showed that although different electrophoretical systems were used, allele frequencies were not significantly different. Thus, the gel system presented has also proved to be ideal for the performance of rapid and sensitive populations studies.

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