EVALUATION OF THE TRIS-GLYCINE/TRIS-CHLORIDE DISCONTINUOUS BUFFER SYSTEM FOR THE ELECTROPHORETIC ANALYSIS OF VNTR AND STR LOCI.

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INTRODUCTION

The analysis of PCR-amplified products by discontinuous polyacrylamide gel electrophoresis followed by silver stain provides high resolution and increased sensitivity when compared with continuous zone electrophoresis in agarose or polyacrylamide gel and ethidium bromide staining [1]. A simple and high resolution, horizontal discontinuous (Tris-borate/Tris-sulfate buffer system) polyacrylamide gel electrophoresis method has been described for AMP-FLP analysis of VNTR loci [2]. More recently, it has been shown that the use of a stacking gel, a wide pH difference between the gel and running buffer, and a low cross-linking agent concentration in vertical discontinuous polyacrylamide gel electrophoresis yields good resolution and sharp DNA fragments in the AMP-FLP analysis [3].
In this article we present a vertical discontinuous polyacrylamide gel electrophoresis method based on the Tris-glycine/Tris-chloride buffer system [4, 5]. The initial zone sharpening of the DNA fragments produced by the glycine-chloride moving boundary is assured by simply using a cap gel above the sample rather than using a stacking gel and an operational shift in the pH. The resolution and reproducibility of this electrophoretic method in the separation of AMP-FLP alleles of two VNTR loci (D1S80 and ApoB) and two STR loci (HUMTH01 and HUMFABP) is evaluated.

MATERIALS AND METHODS

Sample preparation

DNA was extracted from EDTA blood samples by the standard phenol-chloroform extraction procedure.

Amplification

PCR amplification was performed for two VNTR loci (D1S80 and ApoB) and two STR loci (HUMTH01 and HUMFAPB). The primer sequences and PCR time-temperature profiles were as previously described [2, 6, 7].

Electrophoresis

Vertical polyacrylamide gels (16-24 X 14 X 0.075 cm) were prepared by mixing the required amounts of acrylamide and piperazine diacrylamide as cross-linker in a solution containing 0.375 M Tris-Chloride, pH 8.8. A 20 teeth comb (25 mm deep) was used to form the sample wells. After polymerization, wells were washed with 0.375 M Tris-Chloride and filled with the cap gel (0.04% amonium persulfate). The samples (5µl of PCR product mixed with 1 µl of 80% sucrose, 0.2% bromophenol blue in 0.375 M Tris-Chloride) were immediately loaded with a microsyringe into the well bottoms. Electrophoresis was carried out in a HOEFER SE600

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vertical electrophoresis unit at constant current (30 mA) during the first hour and then at constant voltage (55 V) until the bromophenol blue reaches the bottom of the gel. The electrode buffer was 0.025 M Tris-glycine, pH 8.8. After electrophoresis the DNA fragments were silver stained [8]. The composition of the cap gel and the separating gel depending of the marker analyzed were as follows:

	D1S80	ApoB	HUMTH01 and HUMFABP
CAP GEL	7% T, 3% C	6% T, 3% C	8% T, 3% C
SEPARATING GEL	7.5% T, 3%C	6% T, 3% C	11% T, 3% C

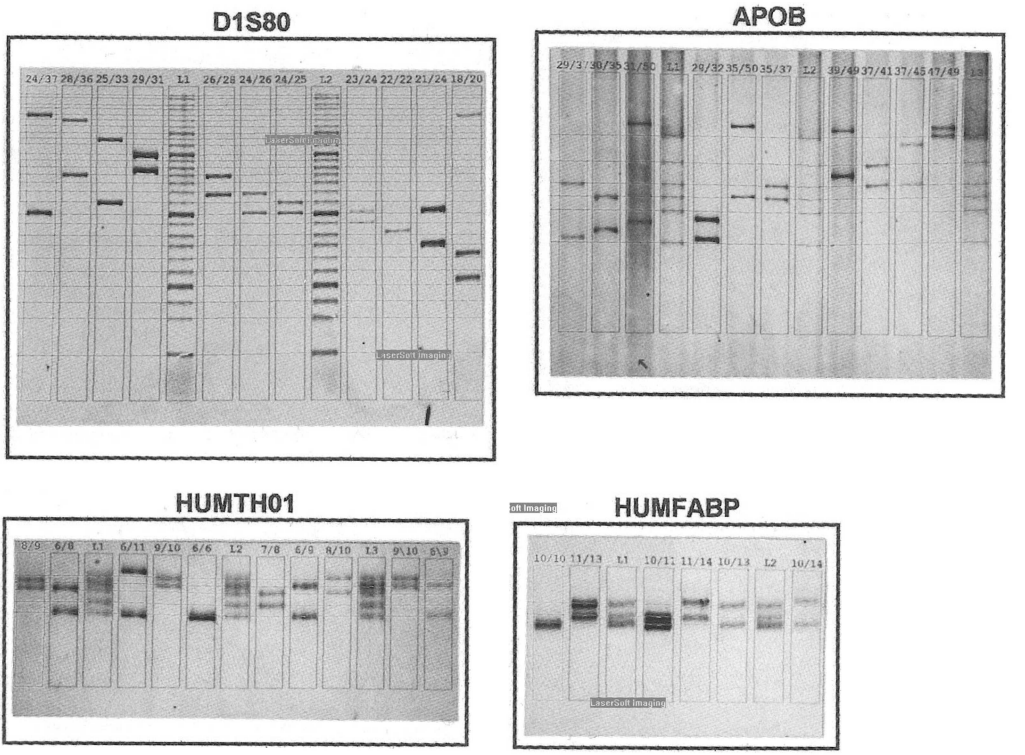

Figure 1. Analysis of two VNTR loci (D1S80 and ApoB) and two STR loci (HUMTH01 and HUMFABP) by vertical discontinuous polyacrylamide gel electrophoresis using the Tris-glycine/Tris-Chloride buffer system.

RESULTS AND DISCUSSION

The cap gel: a simple strategy to assure the initial sharpening of DNA fragments.

The first attempts to separate DNA fragments by vertical discontinuous polyacrylamide gel electrophoresis using the Tris-glycine/Tris-Chloride buffer system were carried out in our laboratory by using homogeneous polyacrylamide gels without stacking gel and loading the samples into the wells filled with the gel buffer (0.375 M Tris-Chloride). Under these conditions

the DNA fragments appeared, in most cases, distorted and diffuse on the silver stained gels. The same results were obtained even when a stacking gel was used (data not shown). It was also observed in these experiments that the glycine-chloride moving boundary formed at the interface of the upper buffer and the top of the gel was distorted when it passes through the sample zone.
By simply filling the sample wells with a cap gel containing the leading ion and underlayering the samples into the well bottoms, the moving boundary was prevented from distortion while passing through the sample zone. This resulted in sharp DNA fragments in the silver stained gels (Fig. 1).

Resolution and reproducibility

As can be seen in Figure 1 the resolution obtained with this electrophoretic system (using a separation distance of only 12-13 cm) could clearly distinguish VNTR alleles ranging in size from 400 to 900 bp and differing by only 16 bp (D1S80) or 15 bp (ApoB) as well as STR alleles of aproximately 200 bp and differing by only 4 bp (HUMTH01) or 3 bp (HUMFABP).
Maximal resolution was achieved when the electrophoresis was carried out at constant current (30-45 mA) until the moving boundary had migrated 1 cm from the bottom of the sample wells and then at constant voltage (50-75 V) overnight.
A comparison of the relative migration of the D1S80 alleles separated at constant current (30 mA) or at constant voltage (55 V) is shown in Figure 2.

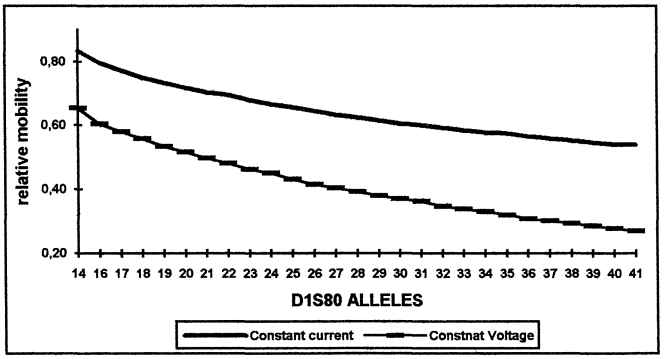

Figure 2. Relative migration vs size of the D1S80 alleles separated at constant current (30 mA) or at constant voltage (55 V).

On the other hand, this vertical electrophoretic system provides a high inter-gel reproducibility and is free from band smiling or waving across the gel. This make very easy the classification of the different alleles by comparison with the control ladders.

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