THE USE OF CAPILLARY ELECTROPHORESIS IN FORENSIC DNA ANALYSIS

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INTRODUCTION

Hypervariabilty in sequences of genomic DNA is to date the most reliable way of personal identification from biological samples. Polymerase chain reaction (PCR) solved many of the problems related to the specimen's quantity and degradation, but the accurate evalutation of the dimension of the amplified bands is still a problem. Horizontal polyacrilamide gels give good results with relatively few samples , but this step of identification can be improved .
We tested a system of capillary electrophoresis in order to gain in accuracy of separation and to reduce the quantity of samples required significantly. Electrophoresis was performed utilizing both U.V.and Laser detector with different fluorescent dyes, buffers and viscous media in order to optimize both resolution and sensitivity.

MATERIALS AND METHODS

DNA Extraction and Amplification

DNA was obtained by phenol-chloroform extraction from bloodstains related to criminal cases. The amplification of the D1S80 locus was carried out according to Budowle et al... To better confirm the results an electrophoretical separation of the PCR products was performed using a high resolution horizontal rehydratable polyacrylamide gel and silver staining as described by Budowle et al..

Capillary Electrophoresis

Electrophoresis were performed on Beckman PACE 2100, either equipped with UV or Laser detector . Capillary was Alltech DB17 40 cm x 100 µ and buffer contained 100 mM Tris borate brought to pH 8,7 with CsOH 1% Hydroxyethyl cellulose. When analyzed in UV ethidium bromide 15 µg/ml was added while in Laser ethidium bromide 500ng/ml plus YOPRO 1 (491/509) 13µg/ml were added.
Samples were run at 9 KV 20°C. All amplified samples were purified in microcon filters (30.000 Daltons exclusion) and loaded diluted 1 to 10.

Results and Discussion

It is apparent the sensitivity of the method which allows to detect bands of 10 ng/ml of DNA and the reproducibility of the analysis which permits to run not only mix between two different samples in order to show the identity of two alleles, but to run together sample and ladder eliminating the possibility of misinterpretation linked to the shift in migration in the sample.
Moreover, a standard amplification sample can be utilized to repeat the analysis at least 20 or 30 times so to give a direct comparison with other samples everytime.
Limitations are to date, the time consuming analysis for single sample (approximately 30 minutes) and the need to optimize the reproducibility of results during the use of the same capillary.
Better results as specificity and sensitivity can be obtained utilizing fluorescent labelled primers or labelled probes, even if in this case new migration problems will be introduced by the single strand-oligonucleotides hybrid.

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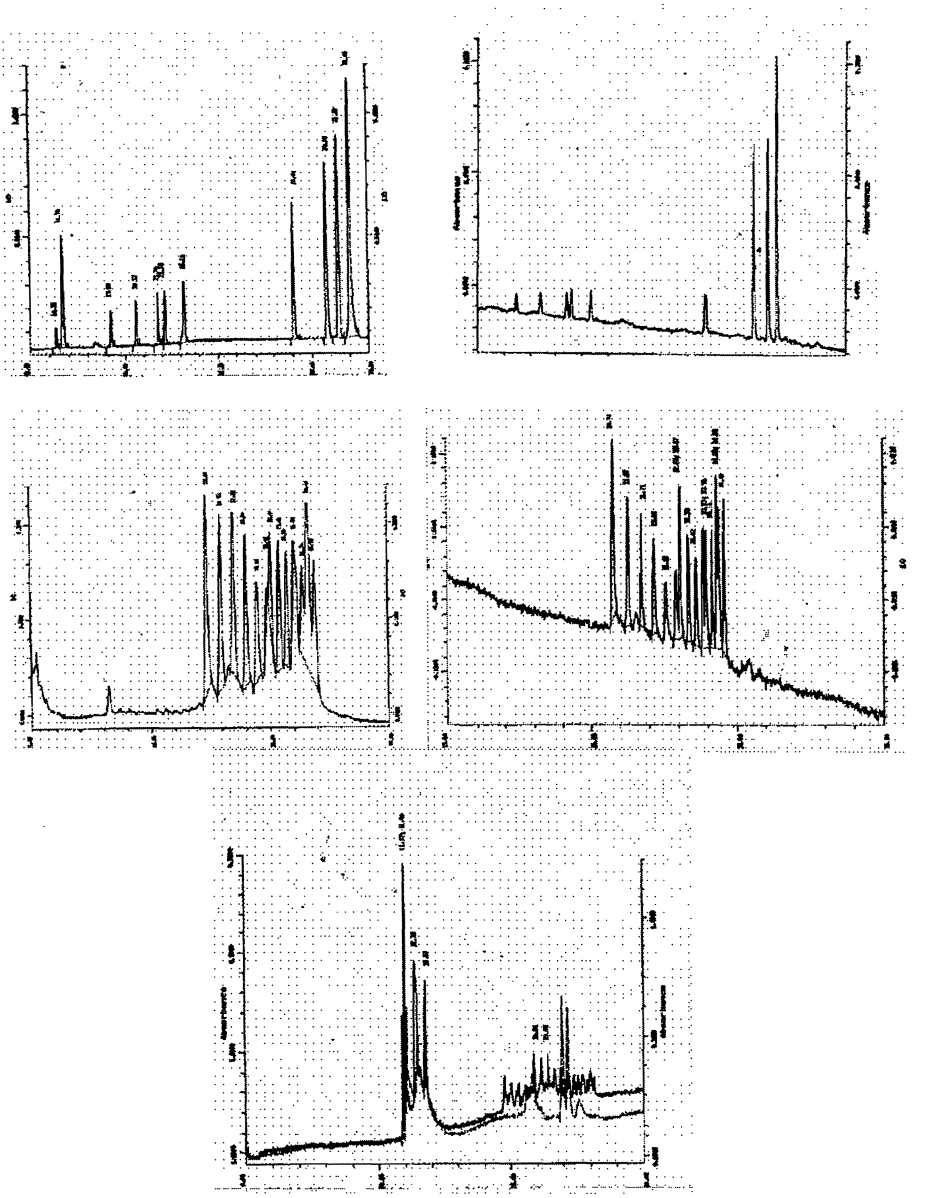

(1) PhiX/Hae III Marker , 5 µl injected at a concetration of 100 µg/ml , UV detection
(2) PhiX/Hae III Marker , 5 µl injected at a concetration of 100 ng/ml , Laser detection
(3) D1S80 Ladder, 5 µl injected , Laser detection
(4) D1S80 Ladder, 5µl injected , after a dluition of 1 to 10 , laser detection
(5) 29/32 D1S80 genotype (dotted) . 5 µl injected after a diluition of 1 to 10, superimposed to the allelic ladder.Laser detection