

1.4 PCR

A METHOD FOR THE ANALYSIS OF PCR AMPLIFICATION PRODUCTS TO IDENTIFY BASE-PAIR SUBSTITUTION AND VNTR POLYMORPHISMS

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INTRODUCTION

A Dot Blot format using allele specific oligonucleotides is commonly used to identify base pair substitution polymorphisms following the PCR amplification of target genomic DNA. A common application of this technique in forensic science has been to identify polymorphisms associated with the DQa region of the major histocompatibility complex (HLA DQa).

A method will be described which permits the identification of multiple base-pair substitution and VNTR polymorphisms using a single technique. The method relies on the simultaneous identification of homoduplexes and heteroduplexes resulting from the PCR amplification of target genomic DNA.

From Genomic DNA isolated from peripheral blood cells target sequences were amplified in a 100 ul reaction mixture containing approximately 300 ng genomic DNA, mixed nucleotides (dATP, dCTP, dGTP and TTP, 1.25 mM each), primers (100 pmol), Taq polymerase (2.5U, Perkin Elmer, Cetus, Emeryville, CA), and 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl at pH 8.3, 1.5 mM MgCl₂, and 0.01% (w/v) gelatin). The primers used to amplify the HLA-DQA locus were SLI-243 (5'-GTGCTGCAGGTGTAACTTGTACCAG) and SLI-244 (5'-CACGGATCCGGTAGCAGCGGTAGAGTTA) using standard amplification conditions.

FORMATION AND DIFFERENTIATION OF HOMODUPLEXES AND HETERODUPLEXES

The validity of this procedure for genotyping was tested using the HLA-DQA polymorphic region. Amplified products from the HLA-DQA locus of different individuals electrophoresed through an agarose gel ran as a single fragment of approximately 240 base pairs, as shown in Fig. 1A. However, when the PCR products were run on a 15% polyacrylamide gel under non-denaturing conditions, several distinct polymorphic patterns were seen for different individuals (Fig. 1B).

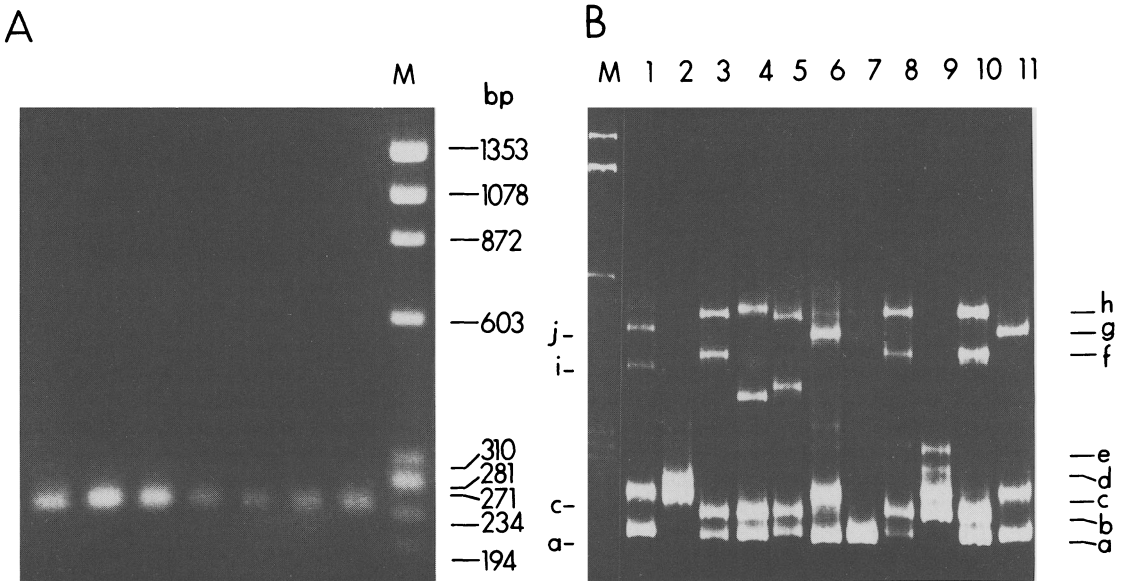


Fig. 1A

Fig. 1B

GENOTYPE ASSIGNMENT

The HLA-DQA genotype represented by each unique polymorphic pattern seen on the polyacrylamide gel were compared with the typing done by dot blot analysis using allele specific oligonucleotide probes. As shown in Fig. 2, amplified products from samples 6-11 of Fig. 1B were analyzed by the dot blot method. The polymorphic patterns associated with samples 9, 10 and 11 were typed as HLA-DQA (1,4), (4,7) and (1,7) respectively.

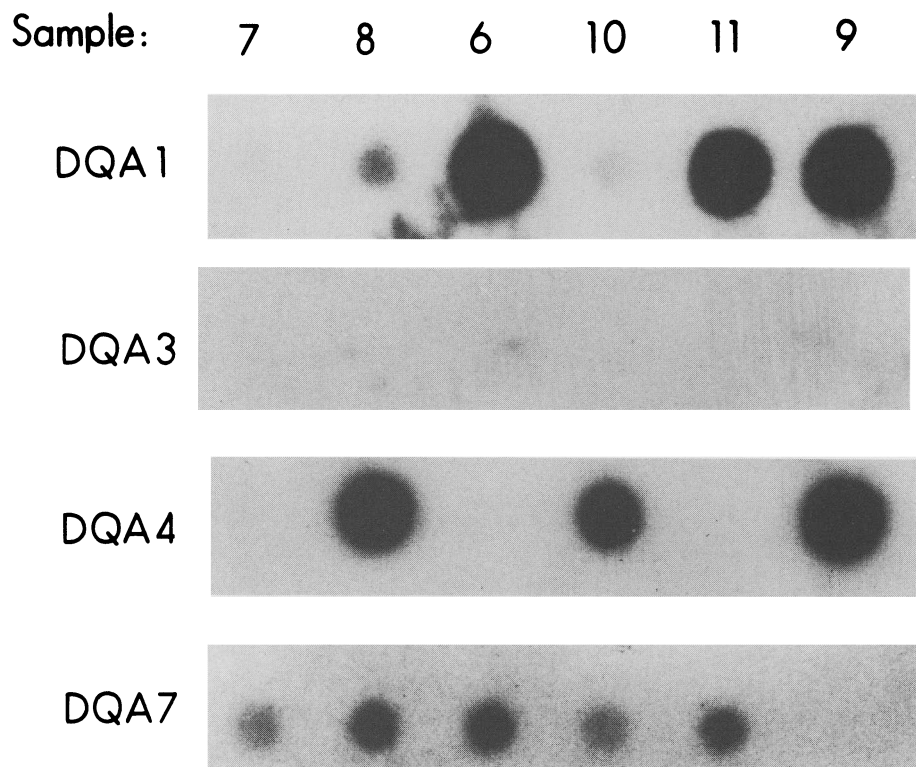


Fig. 2

Additional proof that the pattern resulted from homoduplex and heteroduplex formation is provided by the following experiments. As shown in Fig. 1B, the polymorphic pattern for sample 1 consists of bands a, c, i and j. When band a from sample 1 was reamplified, only band a was obtained (Fig. 3), Lane 1. When band c from sample 1 was reamplified, only band c was obtained, Lane 2. However, reamplification of either band i or band j from sample 1 resulted in all four bands (a, c, i and j,) Lane 3. These results show that bands a and c are homoduplexes while bands i and j are heteroduplexes. Similar results were obtained with sample 11 (Fig. 3).

Sample: 1 11 15
 Band: a c i j a c g k M

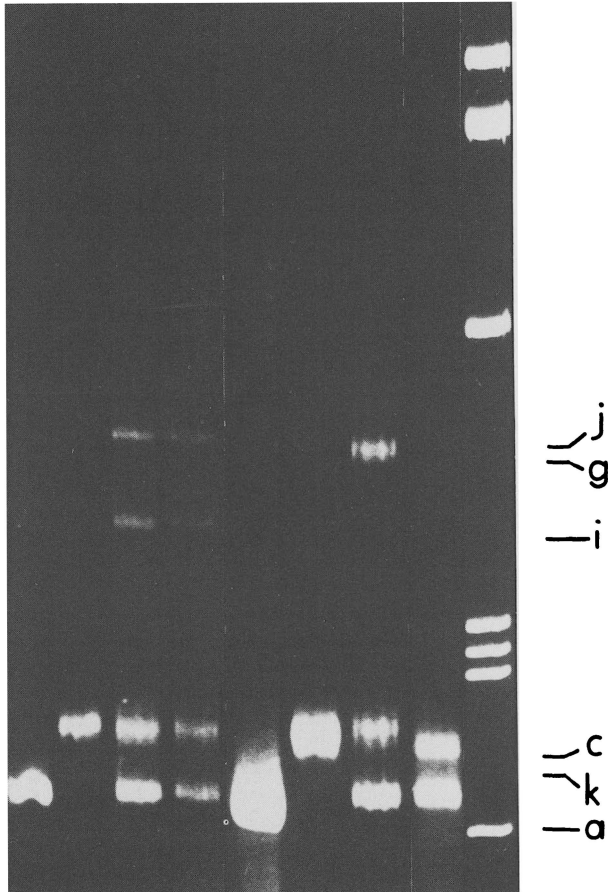


Fig. 3

In addition, sequencing of band b and c from sample 9 (Fig. 1B) confirmed the assignment of homoduplexes and heteroduplexes representing HLA-DQA allele 4 and 1.2 respectively.

APPLICATIONS IN GENOTYPING OF HYPERVARIABLE LOCI

Homoduplexes and heteroduplexes in hypervariable loci were examined. As shown in Fig. 4, polymorphic patterns were obtained when PCR products from the VNTR region associated with the locus 3' to the apolipoprotein B were

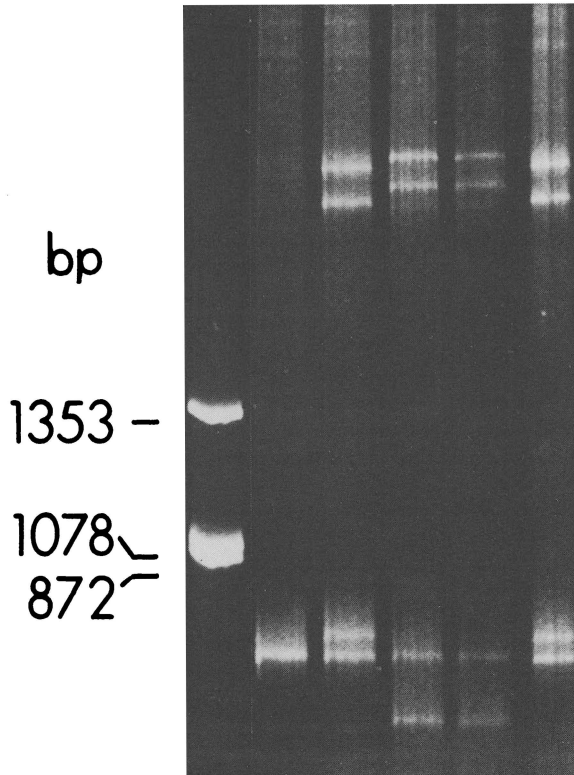
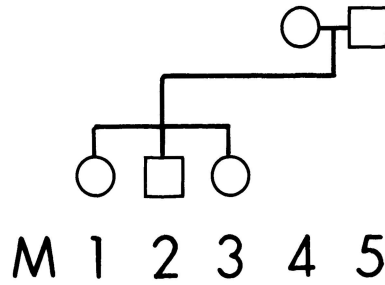


Fig. 4

subjected to polyacrylamide gel electrophoresis. Similar to what has been observed with the HLA-DQA locus, the polymorphic pattern is composed of 1-4 bands. The lower bands are believed to be homoduplexes and the upper bands are formed by interstrand combination of parental homoduplexes, i.e. heterduplexes. Similar results were obtained with the hypervariable region in the 3'-flanking region of the Type II collagen locus. The polymorphic pattern is composed of two to four bands.