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Forensic applications of the genomic matching technique: profiling the alpha block in the major histocompatibility complex

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Abstract. The genomic matching technique (GMT) was developed to characterize polymorphic sequences within the human major histocompatibility complex (MHC), known as polymorphic frozen blocks (PFBs). PFBs are 200–300 kb sequences containing duplications, indels and SNPs. The power of the GMT to differentiate between individuals at the DNA level means that it can be applied as an exclusion tool in forensic science. Here, the GMT is applied to the alpha block in the MHC.

Keywords: Alpha block; Polymorphic frozen block; Ancestral haplotypes; Genomic matching technique; Major histocompatibility complex

1. Introduction

Polymorphic frozen blocks (PFBs) were first observed in the major histocompatibility complex (MHC), and have since been described elsewhere in the genome [1,2]. The sequences within the PFBs are extremely polymorphic with increased numbers of SNPs, indels, and duplications when compared to flanking sequences [3,4]. Haplospecific geometric elements (HGEs) are polymorphic sequences within blocks that can be used as markers for the entire block sequence [4,5]. A profiling technique termed genomic matching technique (GMT) utilises primers that target HGEs, which differ in size, content and number, within the PFBs [4]. Thus, by utilising a single primer set, a complex haplospecific profile may be utilised in forensic science as a cost-effective exclusion tool.

Primer sets have previously been developed for the beta (containing HLA-B) and delta block (containing HLA-DR) of the MHC [6]. Here, the technique is extended to the alpha block (spanning HLA-A to HLA-F) of the MHC.

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2. Methods

Sequence for the alpha block region from HLA-A to HLA-F (AF055066) was plotted against itself using the Dotter program [8]. Priming sites were selected within duplicated regions.

Selected primer sequences were searched against the alpha block regions for the ancestral haplotypes (AHs) 7.1 (PGF-HLA-A*0301; -B*0702; -DRB1*15011) and 8.1 (COX-HLA-A*0101; -B*0801; -DRB1*0301) (http://www.sanger.ac.uk/HPG/Chr6/MHC/index.shtml) using BLASTn (http://www.ncbi.nlm.nih.gov).

DNA samples were taken from cell panels [7] and an internal panel of Caucasian samples.

PCR was performed in a 20 μl volume containing 200 ng DNA, 1.5 mM $MgCl_2$, 1.3 U Taq Polymerase (Fisher Biotec) and 1.25 pmol primers (Falph1 5′CCATGCTG-AGTCTTGTGATAC-3′ and Ralph2 5′ACAGGAAGCTTAAAAACCAGC-3′). The reactions involved 95 °C, 2 min; then 30 cycles of 95 °C, 30 s; 52 °C, 30 s; 72 °C, 1 min; and a final cycle at 72 °C, 1 min. PCR products were visualised as previously described [5,9].

3. Results

Multiple duplications within the alpha block were identified [3] and primers selected within duplicated regions that flanked HGEs.

Priming sites within the 8.1 and 7.1 AHs revealed unique HGEs (Table 1). Products differ in length and content.

Previous studies have shown that samples matched by GMT have concordant HLA typing, and discordant HLA-typed samples are GMT mismatched. Disparity arises between GMT mismatches and HLA matches, indicating that GMT detects greater

Table 1
AHs have unique priming sites and alpha block HGEs

Cell and AH	Product Sequence[a]	Product Size (bp)
COX 8.1 AH	CCATGCTGAGTCTTGTGATACTTATTCCTTTGTATTTCTTCAAGATTTG TGCAATAAG$(TA)_5$ATACA$(TA)_5$TGTAT$(GT)_3(AT)_5$ATCCCCAAAACAAAT ATCAGTTTGTTTGCTGGTTTTTAAGCTTCCTGT	153
	CCATCCTGAGTCTTCTGATACTTATTCCTTTGTATTTCTTCAAGATTTGT GCAATAAG$(TA)_{20}$TCTCCCAAAACAAATATCAGTTTGTTTGCTGGTTTTT AAGCTTCCTGT	149
	CCATGCTGAGTCTTGTGATACCTTATTCCTTTGTATTTCTTTAAAGATT TGCACAATAAG$(TA)_6$TGTAC$(AT)_5$CTGAAAAAAAATTATTTGCTGGTTT TTAAGCTTCCTGT	129
PGF 7.1 AH	ACAGGAAGCTTAAAAACCAGCAAAACAAACTGATATTTGTTTGGGG(A T)$_6$(AC)$_3$ATAC$(AT)_5(GT)_5(AT)_3$ACA$(CATA)_2$TACACA$(TA)_3$C$(AT)_3(GT)_2$(AT) $_3(AC)_2(AT)_3$AC$(AT)_3$GGATAC$(AT)_3(GT)_2$(ATATATACAC)$_2(AT)_3$GTAT(ATA TATACAC)$_2(AT)_3(GT)_2(AT)_4$ACTTATTGCACAAATCTTTGAAGAAATACA AAGGAATAAGTATCACAAGACTCAGCATGG	296[b]
	CCATCCTGAGTCTTCTGATACATATTCCTTTGTATTCCTTCAAAGATTT GTGTCAATAA$(AT)_9$CTCCCCAAAACAAACCTCAGTTAGTTTGCTGGTTT TTAAGCTTCCTGT	124

[a]Primer sequences boxed.

[b]Primers acting in the opposite orientation to all other products.

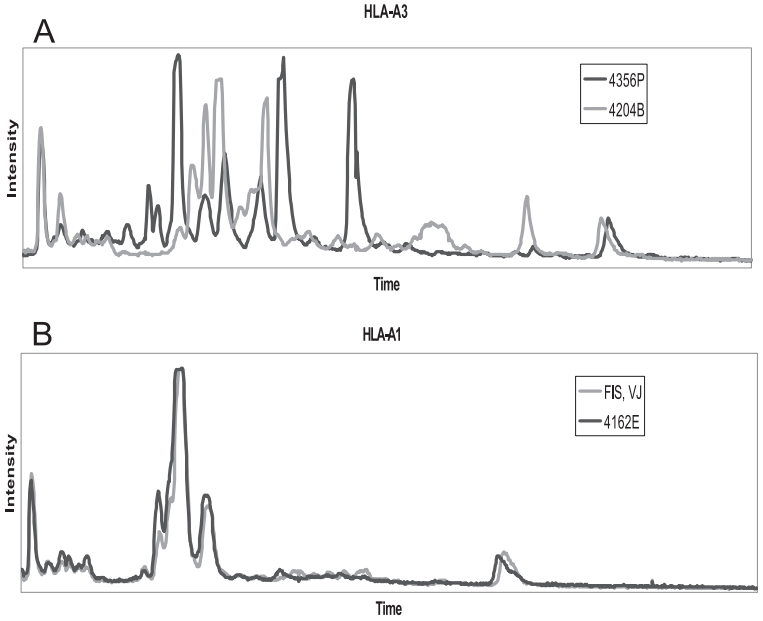

Fig. 1. Alpha block profiles are more discriminatory than HLA-A typing alone. (A) Samples typed as HLA-A3 but with different alpha block GMT profiles. (B) Samples with same HLA-A typing and alpha block profile. Horizontal axis is a relative time scale as products move through the gel and vertical axis is intensity of ethidium bromide fluorescence. First peak in profile corresponds to 59 bp internal standard.

polymorphism than HLA typing alone [9]. Seventy-two samples profiled at the alpha block produced results supporting these findings (Fig. 1).

4. Discussion

The application of the GMT has been shown to characterize polymorphism within the alpha block of the MHC, more so than HLA-A typing alone. Multiplexing for PFBs within the genome will produce economical, individual specific profiles, which can be utilised in forensic science as a powerful exclusion tool.

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