

Analysis of the DNA flanking the hypersensitive site(s) 35332 (G/Gs) (158) were carried out on 210 healthy, unrelated Malaysian individuals (71 Chinese, 70 Malays, and 70 Japans). Genomic DNA from each blood sample was extracted by using the standard preparation of genomic DNA protocol.

Five polymorphic sites (G2, *Hinf*I, *Hpa*II, G1, and *Hpa*I) were assayed for each DNA sample by using the PCR-based restriction enzyme digestion test and electrophoresis. All the 5 sites, only the *Hinf*I, *Hpa*I, and *Hpa*II sites were polymorphic whereas the G1 and G2 were non-polymorphic. For the *Hinf*I, *Hpa*I, and *Hpa*II sites, the alleles appeared to be at Hardy-Weinberg equilibrium, and their allelic frequencies, percent of discrimination (PI), and heterozygosity (H) were calculated. Haplotype frequencies of *Hinf*I, *Hpa*I, and *Hpa*II sites, approximately 67% of the 210 individuals were heterozygous at one or more of the flanking polymorphic sites and the 4 alleles have their single alleles mapped. The *Hinf*I, *Hpa*I, and *Hpa*II sites were mapped to polymerase chain reaction (MVR-PCR).

## Abstract

Five polymorphic assays were tested out only on the flanking sites on genes *Hinf*I, *Hpa*I, *Hpa*II, *Hpa*I, and *Hpa*II individuals by using the PCR-based restriction enzyme digestion, and gel electrophoresis. Statistical analyses for all haplotypes of *Hinf*I, *Hpa*I, and *Hpa*II in the Malaysian and each ethnic group showed that a significant association was observed in all three sites. Significant association was also observed between each pair of the polymorphic sites (*Hinf*I-*Hpa*I, *Hinf*I-*Hpa*II, and *Hpa*I-*Hpa*II). Haplotype G+C appeared to be at the highest frequency, whereas haplotypes C-T and C-C, C+T did not exist in 80 samples and the Chinese population.

Pairwise comparisons of the distribution of 4 haplotypes between different population samples showed that the Chinese, Japanese, and Malays were closely related to each other genetically. The results also indicated that the Chinese shared a great degree of genetic affinity with the Japanese, whereas the other races were not.

Direct DNA sequencing method with an allele-specific primer 35332 (G/G) and a

Analyses of the DNA flanking the hypervariable minisatellite MS32 (locus D1S8) were carried out on 210 healthy, unrelated Malaysian individuals (70 Chinese, 70 Indians, and 70 Malays). Genomic DNA from each blood sample was extracted by using the rapid mini-preparation of genomic DNA protocol.

Five polymorphic sites (O2, *Hinf*I, Hump1, O1, and Hump2) were assayed for each DNA sample by using the PCR-based tests, restriction enzyme digestions, and gel electrophoresis. Of the 5 sites, only the *Hinf*I, Hump1, and Hump2 were polymorphic, whereas the O1 and O2 were monomorphic. For the *Hinf*I, Hump1 and Hump2 sites, each appeared to be at Hardy-Weinberg equilibrium, and their allele frequencies, power of discrimination (Pd), and heterozygosity (h) were calculated. Based on the data of *Hinf*I, Hump1, and Hump2 assays, approximately 67% of the Malaysian individuals were heterozygous at one or more of the flanking polymorphic sites, which can therefore have their single alleles mapped by the allele-specific minisatellite variant repeat mapping by polymerase chain reaction (MVR-PCR).

Haplotype assays were tested out only on the double heterozygous (*Hinf*I-Hump1, *Hinf*I-Hump2, and Hump1-Hump2) individuals by using the PCR-based tests, restriction enzyme digestions, and gel electrophoresis. Statistical analyses for all haplotypes of *Hinf*I, Hump1, and Hump2 in the Malaysian and each ethnic group showed that a significant association existed among these three sites. Significant association was also observed between each pair of the polymorphic sites (*Hinf*I-Hump1, *Hinf*I-Hump2, and Hump1-Hump2). Haplotype G+C appeared to be at the highest frequency, whereas haplotypes C-T and C-C, C+T did not exist in all samples and the Chinese, respectively.

Pairwise comparisons of the distributions of 8 haplotypes between different population samples showed that the Chinese, Indians, and Malays were closely related to each other genetically. The results also indicated that the Chinese shared great degree of genetic affinity with the Japanese, whereas the other races were not.

Direct DNA sequencing method with an allele-specific primer 32-H1C and a

universal primer 32-O was used to sequence the 5' flanking region in 12 samples. A total length of 244 bases was obtained by this approach, in which the allele-specific primer 32-H1C was used to amplify the DNA strand with H1<sup>C</sup>, and then separated from the DNA strand with H1<sup>G</sup> in the homologous chromosome. The known polymorphic sites (*Hinf*I, Hump1 and Hump2) remained the same and no new polymorphic sites were detected.