Direct haplotyping of chromosomal segments from multiple heterozygotes via allele-specific PCR amplification

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Allele specific amplification (ASA) of a DNA segment [1] is possible if the polymorphism defining the allele is at the 3' end of one of the two primers required for polymerase chain reaction (PCR) [2]. The presence or absence of product after amplification with a given allele-specific primer and an invariant primer annealing distally types the polymorphic priming site. Beyond its discrimination at the polymorphic site, we hypothesized that ASA would selectively amplify the segment encompassed between primers in either the paternal or maternal chromosome of a heterozygote. Hence, it should be possible to haplotype directly all polymorphisms nested between PCR primers.

We characterized a pedigree (Fig 1a) at two polymorphic loci in the β -globin cluster [3]. One locus was typed 1/3 according to presence or absence of a 630 bp product after ASA with allele-specific primers GR1/GR3 and invariant primer GR5 (Fig 1b). The second locus, nested within the PCR segment, was typed +/- for a TaqI site using a restriction fragment length polymorphism (RFLP) on product amplified with GR5 and primer "GR=" (Fig 1c). "GR=" anneals indiscriminately to the GR1/GR3 site. Homozygote "A" sets the phase of one chromosome in "B" as 1,-; the other chromosome is 3,+ which is inherited by "I" (homozygous 3,+) through "G". Therefore, the haplotypes are 1,- and 3,+.

We analyzed all heterozygotes by digesting their GR1 or GR3 ASA products with TaqI (Fig 1d). Whereas product amplified with "GR=" is heterozygous, GR1 ASA product is hemizygous for the upper band (-) and GR3 ASA product is hemizygous for the lower band (+). Thus, the haplotypes can be derived directly, 1,- and 3,+, in agreement with pedigree analysis.

ASA should allow direct determination of haplotypes encompassed between primers without need for pedigrees if allelic sequences are known at the polymorphic priming site. It should be useful in population and clinical genetics and in haplotyping highly polymorphic regions such as HLA-DQ α [4].

FIGURE 1

Pedigree (a) typed for two polymorphisms in the inter-Alu segment between ψ and δ globin genes [ref 3] using ASA (b) and RFLPs (c). ASA product from "B", "D", "G", "H" was RFLP typed to determine haplotypes directly (d). **METHODS:** ASA GR1 and GR3 anneal to a site having a polymorphic TG deletion near their 3' end. GR3 and GR1 are complementary, respectively, to deletion and non-deletion sequences. PCR (55°C annealing) with GR1/GR5 and GR3/GR5 yielded an allele-specific 630 bp product. [No product was amplified in the empty lanes, as probed with labelled GR5. Higher annealing temperatures reduced yield considerably, lower ones amplified non-specifically.] RFLP Non-discriminating "GR=" and GR5 were used for PCR (50°C annealing). Product digested with 10 U TaqI yielded bands of 540 (-) or 430 (+) bp. **ASA/RFLP** TaqI digest of "GR=", GR1 and GR3 products. **PRIMERS (5' to 3')** GR1 GCTTTTCAC(TG)TCA; GR3 AGCTTTTCAC(TG)TCAA; "GR=" TTCAC(TG)TCAAAATCTAG; GR5 AAGTGGAAAGATAGCATCT [ref 3]. **PCR** 30 cycles [denaturing 94°C (1'); annealing (2'); polymerization 72°C (2')] in 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris pH 8.4, 200 μ M each deoxynucleotide, 1.5 U Taq polymerase, 0.1 μ M each primer and 1 μ g genomic DNA. 10% (b,c) and 15% (d) of products were electrophoresed in a 2.5% agarose gel, stained and photographed. Lanes with " contain 770 and/or 500 bp markers.

REFERENCES

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