

RAPID DETECTION OF ALLELE-SPECIFIC MINISATELLITE VARIANT REPEAT MAPPING AT D1S8 (MS32) WITH FLUORESCENT LABELED DNTPS

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A novel assay system was devised for allele-specific minisatellite variant repeat mapping using polymerase chain reaction (MVR-PCR) at D1S8 (MS32) without southern blot hybridization. At three polymorphic sites (H1, Hf and H2) in the flanking region, allele-specific MVR-PCR was performed using dCTP[R110] for a-type, dUTP[R110] for t-type, and three sets of allele specific primers (H1G/C, Hf2+/- and H2C/T). Using primers H2C, H2T and Hf2-, about 50 rungs of the MVR ladder could be correctly mapped. Even though in the most distant polymorphic site (H1 site, approximately 400 bp from the first repeat), primer H1C made it possible to show about 40 rungs of the MVR ladder. However, in case of using primer Hf2+, only faint rungs were observed because the primer was probably consumed for dimer formation and the primer dimer became the predominant product. Using primer H1G, a non-specific broad band appeared around the position of 20th rung and disturbed further mapping. In order to improve MVR mapping results, we designed new primers. As the result of investigating the sequences of each primer by computer analysis, primer H1G was indicated to form a primer dimer with primer 32-TAG-A/T, while we found the strong possibility of primer Hf2+ to interact primer TAG. Therefore, we designed two new primers named H1G20A and Hf2+15C by replacing a base in the middle of each primer to prevent primer dimer formation and to remain the allele specificity. We could correctly mapped up to 35 and 40 rungs by these primers. Additionally, we obtained not only enough allele codes but also more than 45 diploid codes using the standard flanking primer (32-D). This novel method for MVR analysis can be applied to the field of forensic and population genetics.