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Modification of enzymatically amplified DNA for the detection of point mutations

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The detection of genetic polymorphisms[1] or point mutations[2,3], correlated with diseases, in enzymatically amplified DNA sequences (Polymerase Chain Reaction), was performed by digestion of PCR products when restriction site exist at least in one allele of the amplified sequence. Until now the polymorphisms[4] or point mutations[5] without restriction site were detected using allele specific radiolabeled probes.

Modified primers can introduce base substitution adjacent to the codon of interest and create an artificial restriction site with only one allelic form (wild or mutated). This approach allowed us to screen for point mutations of codon 12 of Kirsten ras oncogene. After DNA extraction, we performed PCR with a modified primer creating a Msp I recognition site only if codon 12 is of the wild type (fig. 1). The PCR product (99 bp) was digested by Msp I enzyme which gives two fragments of 21 and 78 bp in the case of a wild type sequence or leaves the product undigested in the case of a mutated sequence. The fragments were resolved by electrophoresis on NuSieve agarose gels. This approach is presented in figure 2: 50A is a DNA with two wild type alleles (lanes A and B), SW80 has 2 mutated alleles at codon 12 (C and D) and SG3d (F and G) has a wild and a mutated allele as confirmed by allele specific radiolabeled probes. The electrophoreses are in agreement with the known genotype of these DNAs.

Our approach coupled with a rapid method of DNA preparation (e.g. boiling extraction) allows a fast detection of specific polymorphisms or point mutations in a given gene, and the analysis can be performed within a day. With an automated device for PCR one can test simultaneously many samples and screen for various single locus anomalies. Moreover we can detect asymptomatic heterozygous carriers of recessive genetic diseases even in the absence of a natural mutation related restriction site. This method does not need radioactive compounds and is easier and safer to perform in a large scale than the detection by allele specific radiolabeled probes.

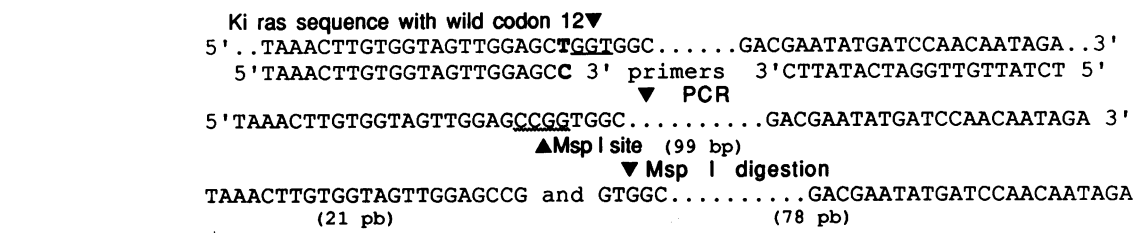

Figure 1

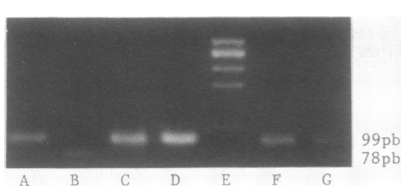

Figure 2: 1 µg of DNA from 50A (A and B), SW80 (C and D) and SG3d (F and G) was amplified by PCR (40 cycles) using the primers described in figure 1. 20 µl of the amplified samples were digested with Msp I (B, D and G) and electrophoresed on a 3% NuSieve agarose gel. Lane E: 2µg of the ΦX 174/Hae III molecular weight marker.

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