Amplification of a highly polymorphic VNTR segment by the polymerase chain reaction

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Submitted January 30, 1989

Some segments of the human genome exhibit polymorphism due to a variable number of tandem repeats (VNTR). Analysis of these VNTR regions is extremely useful in genetic linkage studies, paternity testing, and forensic identification (1).

The VNTR segment detected by probe pYNZ22 (HGM locus D17S30) is one of the most highly polymorphic regions yet identified (2). Over ten alleles have been observed by Southern blotting, with a heterozygosity of 86%. Besides its usefulness as a polymorphic marker, this probe is also closely associated with the Miller-Dieker syndrome (3), and is deleted in over 60% of breast cancer tumors (4).

The recent determination of DNA sequence flanking pYNZ22 (5) suggested that this locus might be analyzed by the polymerase chain reaction (PCR), where specific regions of the genome are amplified by repeated application of synthetic DNA primers and a heat-stable DNA polymerase (6). Of concern, however, were the difficulties encountered when amplifying other VNTR loci (7).

Avoiding segments partially homologous to the repeat unit, we designed two oligonucleotide primers to direct amplification across the pYNZ22 region. PCR products with sizes expected for this locus were observed from 1 μg of genomic DNA after 28 cycles of amplification with primer annealing at 55°C (Fig. 1). The products ranged from 170 bp to 870 bp, as expected for 1 to 11 repeat units between the primers. These alleles showed a Mendelian pattern of inheritance (lanes 2 to 6). A PCR product of 1250 bp (lane 10), apparently unrelated to the pYNZ22 polymorphism, can be suppressed by using less primer and more total dNTP in the amplification.

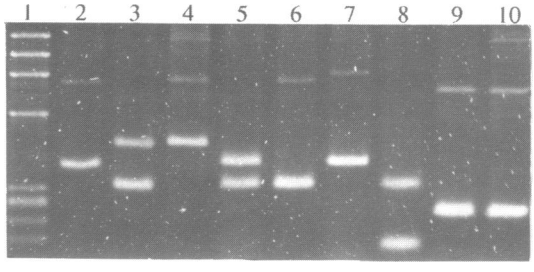

Figure 1. PCR Amplification of the pYNZ22 VNTR. Reactions and gel analysis were performed as described (6), but with 1.1 mM of total dNTP and 0.5 μM of the primers 5'-CGAAGAGTGAAGTGCACAGG-3' and 5'-CACAGTCTTTATTCTTCAGCG-3'. Lane 1: ϕX174/HaeIII marker fragments; lanes 2-6: amplification of two parents and three offspring; lanes 7-9: amplification of three unrelated individuals; lane 10: same as lane 9 except with 1.0μM primers and 0.8 mM total dNTP.

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