

Rapid PCR analysis of the St14 (DXS52) VNTR

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Introduction: Some segments of the human genome exhibit polymorphism due to a variable number of tandem repeats (VNTR). The enzymatic amplification of VNTR loci can allow for rapid analysis and discrimination of closely sized alleles. We report a PCR-based method for analysis of the highly polymorphic St14 VNTR.

Source and Description: The St14−1 probe is a 3 kb EcoRI fragment cloned from human DNA which detects several polymorphisms at the DXS52 loci, including a highly polymorphic VNTR RFLP revealed by TaqI. Analysis of this VNTR by Southern transfer has revealed ten alleles ranging in size from 3.4−6.6 kb, with alleles as large as 15 kb in Black individuals (1, 3). The region responsible for the TaqI polymorphism was sequenced from a cloned allele, and was found to be an almost perfectly duplicated 60 bp repeat (2). To assay the VNTR by PCR, amplification primers were designed on either side of the repeat. However, due to additional repetitive sequence flanking the hypervariable region, the PCR primers were not placed directly adjacent to the VNTR. As a result, amplified products include about 650 bp of flanking sequence.

PCR Amplification: We used 1 μM in each of the primers 5'GGCATGTCATCACTTCTCTCATGTT-3' and 5'-CACC-ACTGCCCTCACGTCACTT-3', 'standard' PCR buffer and dNTP concentrations (4), 1 μg genomic DNA, and 24 cycles of PCR consisting of 20 sec at 94°C, 30 sec at 55°C, and 20 sec at 74°C, with a final incubation for 5 min at 74°C. Less favorable results were obtained with additional cycles. Size differences between the amplified products were found to correspond with those seen on Southern blots; Mendelian inheritance of the allelic products was also verified (data not shown).

Frequency: 50 unrelated Caucasian males were analyzed using the above primers. The amplified allele sizes and frequencies observed are: 3000 bp (2%), 2900 (8%), 2400 (12%), 1690 (36%), 1630 (2%), 1570 (14%), 1390 (10%), 1300 (2%), 1220 (2%), and 700 (12%). We have also seen other rare allelic products (880, 1750, 1810 and 2100 bp) that were not present in these 50 samples. All of these products (except for the 880 bp product) are shown below. The size of the smallest product (700 bp) suggests that it represents an allele with just one 60 bp repeat; and the most frequent product of 1690 bp appears to contain 17 tandem repeats.

Chromosomal Location: The DXS52 loci map to Xq26−28, and are linked to several disease genes including hemophilias A and B, fragile X syndrome, and adrenoleukodystrophy (2). The St14 VNTR specifically maps to Xq28 and is about 2 cM from the hemophilia A-coagulation factor VIII locus, yet its large number of alleles makes it very useful in the diagnosis of hemophilia A.

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REFERENCES

1. Oberlé *et al.* (1985) *N. Eng. J. Med.* **312**, 682−686.
2. Mandel *et al.* (1986) *Cold Spr. Harb. Symp. Quant. Biol.* **51**, 195−203.
3. Barjon *et al.* (1989) *Nucl. Acids Res.* **17**, 2149.
4. Saiki *et al.* (1988) *Science* **239**, 487−491.

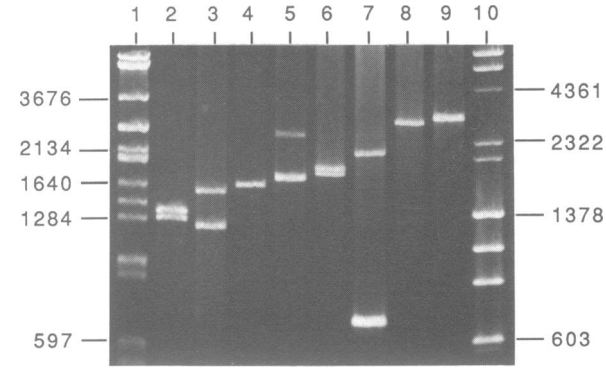

Figure 1. Agarose gel electrophoresis of allelic products from the St14 VNTR locus. PCR products were analyzed on an ethidium bromide stained 1% agarose gel run in 1×TBE buffer at 10 V/cm. The size markers and products shown are: lane 1, AvaII digested phage λ DNA; lanes 2−9, St14 VNTR amplified products: 1300 and 1390, 1220 and 1570, 1630, 1690 and 2400, 1750 and 1810, 700 and 2100, 2900, and 3000 bp; lane 10, HindIII digested phage λ DNA mixed with HaeIII digested φX174 RF DNA.

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