Detection by PCR of a VNTR polymorphism at D4S43

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Source and Description: The anonymous DNA probe pKP1.65 reveals a VNTR polymorphism, detected as a *Stu*I RFLP, within the human D4S43 locus (1). We have sequenced a 1269 bp *Pst*I fragment located immediately adjacent to this probe in order to develop PCR primers for the VNTR (EMBL accession number X60679). A 14 bp G/C-rich core (GGGGAGGGGGAAGA) was found to be imperfectly repeated 6 times in a region approximately 350 bp from one end of this fragment.

PCR Amplification: Initial attempts to amplify this region by PCR led to products of an unexpected size. We speculate that either the G/C-rich nature of this region or alternate DNA structures produced during PCR were interfering with the amplification process. However, the use of 7-deaza-dGTP (2) led to an efficient yield of polymorphic PCR products in the expected size range. DNA amplification was performed with 0.4 μ M in each of the primers GH437 (5'-GACCACAGAGAGCTTAGTGGAGC-TT-3') and GH436 (5'-GACCACTTCACTGACATCCACATC-T-3'), and 'standard' buffer and dNTP concentrations (3), except that 60% of the total dGTP was 7-deaza-dGTP (Pharmacia #27-2090-02). Genomic DNA (1 μ g) was amplified for 28 cycles using 20 sec at 94°C, 20 sec at 55°C, and 20 sec at 74°C, with a final incubation of 5 min at 74°C. The relative sizes of the PCR products were found to correspond with the classes of fragments seen in Southern blot RFLP analysis, and co-dominant segregation of the alleles was verified in two- and three-generation families (data not shown).

Frequency: Genomic DNAs from 81 unrelated Caucasian individuals were analyzed using this protocol. The observed allelic products were as follows (with a PIC value calculated to be 0.77):

Repeats	Size (bp)	Frequency
K1 22	478	.01
K2 14	366	.01
K3 13	352	.03
K4 12	338	.04
K5 11	324	.16
K6 10	310	.07
K7 9	296	.04
K8 8	282	.02
K9 7	268	.31
K10 6	254	.07
K11 1	184	.25

Comments: The D4S43 locus maps to 4p16.3, very close to the lesion responsible for Huntington's disease (1). Amplification with 7-deaza-dGTP at fractions ranging from 20% to 80% of the total dGTP also led to the expected products. Although the amplification as described is specific for this locus, a heteroduplex of the allelic products is often visible with heterozygous samples as an upper third band (Figure 1).

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References: 1) MacDonald *et al.* (1989) *J. Clin. Invest.* **84**, 1013-1016. 2) McConlogue *et al.* (1988) *Nucl. Acids Res.* **16**, 9869. 3) Saiki *et al.* (1988) *Science* **239**, 487-491.

Figure 1. Agarose gel electrophoresis of allelic products from the D4S43 VNTR locus. PCR products were analyzed on an ethidium-stained 2% agarose gel run in 1×TBE at 10 V/cm. Lanes 3 and 6 show some of the Φ X174/*Hae*III marker fragments, with sizes indicated on the right. The allelic products in each lane, as designated by the number of repeats, are; lane 1 (1, 6), lane 2 (7, 11), lane 4 (8, 9), lane 5 (10, 11), lane 7 (12, 13), lane 8 (7, 14). Prominent heteroduplex bands are visible above the allelic products in lanes 2, 4, 5, and 7.