Dinucleotide repeat $(TG)_{23}$ polymorphism in the MAOB gene

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Source/Description: A 0.9kb TaqI restriction fragment (designated MAOBTG-1) was isolated from the human genomic MAOB clone p35.B1b (1) and sequenced. The dinucleotide sequence $(TG)_4$ CCTC$(TG)_{23}$AGAC$(AG)_4$ is located 260 bp downstream from the exon 2 donor site. Southern blot analysis of MAOA genomic clones with a MAOA(CA) dinucleotide repeat probe (2) suggests that like MAOB the MAOA repeat is also located in intron 2.

Polymerase Chain Reaction (PCR): The following flanking sequence primers will amplify fragments ranging from 197 to 207 bp:

TG strand: 5′ CTTCACAGCCTCTCTCCCAG 3′

AC strand: 5′ CTTCCTATTTCTCTCTCTGTC 3′

Optimum conditions for amplification are an initial cycle of 94°C for 5 min, 50°C for 1 min, 72°C for 2 min followed by 33 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 3 min with a final elongation step of 10 min at 72°C. Reaction volumes of 50 µl contain 30–100 ng of genomic DNA, 100 ng of each primer, 500 µM each of dGTP, dCTP, and dTTP, 6.3 µM dATP, 3.8 µCi α^{32}P-dATP (3000 Ci/mmol), 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, pH 8.3 and 2.5 units Taq polymerase.

Frequency: Estimated from 64 chromosomes (20 male; 22 female)

Allele	Length (bp)	Frequency
A1	197	0.125
A2	199	0.297
A3	201	0.063
A4	203	0.141
A5	205	0.359
A6	207	0.015

Observed Heterozygosity: 63.6%; PIC = 0.73.

Chromosomal Location: The MAOB gene has been assigned to Xp11.23-Xp22.1 by in situ hybridization (3).

Mendelian Inheritance: X-linked, codominant inheritance was observed in four two- and three-generation families (n = 32). The PCR amplified sequence of MAOBTG-1 has been submitted to EMBL. Accession no. X63276.

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Dinucleotide repeat polymorphism at the D5S98 locus

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Source and Description: A human genomic cosmid library was constructed in vector Lorist B and screened with the probe ECB27 (D5S98) (1). Two clones were isolated and both were shown to contain the same $(C\text{-}A)_n$ repeat when hybridized to an end labelled $(G\text{-}T)_9$ oligonucleotide.

Primer Sequence:

Oligo. I 5′-GTATTCTTTTACTTGCTTAGAGCCT-3′

Oligo. II 5′-GTCTATATGCTTGTTACAGATGTTC-3′

Polymorphism and Frequency: Two alleles only were detected in 33 individuals tested.

Allele	Number of C A repeats	Frequency	Product size (bp)
B1	10	0.61	117
B2	9	0.39	115

Mendelian Inheritance: All the DNAs amplified had already been typed for the BglII polymorphism detected by the probe ECB27 (1). The CA repeat polymorphism showed complete linkage disequilibrium to the BglII polymorphism in 20 UK families.

Clinical Relevance: Presymptomatic diagnosis of adenomatous polyposis coli (APC) (2).

Chromosomal Localization: Assigned to chromosome 5q21 by fluorescent in situ hybridisation (3).

PCR Conditions: Reactions were carried out in a total volume of 100 µl containing: 1 µg genomic DNA, 50 pmol of each oligo, 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 6.7 mM $MgCl_2$, 1 mM β-mercaptoethanol, 6.7 mM EDTA, 1.7 µg bovine serum albumin, 150 µM dATP, dGTP and dTTP, 0.6 µm dCTP, 1.5 µCi (α-^{32}P) dCTP (Amersham, 3000 Ci/mmol), 1 unit Taq polymerase (Anglian Biotechnology). Amplification was for 30 cycles with denaturation at 93°C for 30 sec, annealing at 46°C for 30 sec and extension at 70°C for 30 sec. Amplified products were resolved on DNA sequencing gels and detected by autoradiography after overnight exposure.

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