Poly (T/A) polymorphism at the human retinal degeneration slow (RDS) locus

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Source/Description: We have designed PCR primers flanking a $(T/A)_{19}$ repeat based on the published nucleotide sequence of the Human *RDS* cDNA. These primers allow the amplification of a 96 bp fragment from nucleotide 1705 to 1800 in the 3′ untranslated region of the gene (1).

PCR Primers:
RDS1 5′ CACTTGGTGCATAAGCACAGA 3′ -A strand
RDS2 5′ AAGCAAACGGCCAACCTGTCA 3′ -T strand

Allele Frequencies: Estimated from 160 chromosomes of unrelated CEPH family parents and 24 chromosomes from unrelated Irish individuals. PIC = 0.57.

Allele (bp)	Frequency
D1 98	0.49
D2 97	0.20
D3 96	0.31

Chromosomal Location: The human *RDS* gene has been previously mapped to 6p12 by in situ hybridization and somatic cell hybrids (1). Linkage analysis of *RDS* with other markers on 6p using 40 CEPH families has provided further evidence of localization to this region:

	θ	Z
HLA-B	0.18	26.25
D6S19	0.09	17.46
KRAS	0.19	13.11

Mendelian Inheritance: Codominant inheritance observed in 40 CEPH families.

Protocol: PCR was carried out as described previously (2), except: samples were heated at 94°C for 4 min and then processed through 30 cycles consisting of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C.

Other Comments: We have observed no recombinations between this marker and a previously unmapped Autosomal Dominant Retinitis Pigmentosa locus (RP6) (Z_m = 5.38, θ = 0.00) (reported in a parallel communication (3)). Hence this marker will be extremely useful for linkage studies of this retinopathy.

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PCR detection of a TaqI polymorphism at the CCAATT box of the human tyrosinase (TYR) gene

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Description/Methods: The 5′ promoter region of the human tyrosinase gene (TYR) was amplified with the polymerase chain reaction (PCR) and digested with TaqI to detect a two allele polymorphism at the CCAATT box site (nucleotide −199) (1). This TaqI polymorphism is the second of two different TaqI polymorphisms reported for the tyrosinase gene (2). Thirty five cycles of PCR were performed in 100 μl volumes containing 1–2 μg genomic DNA, 1 μM of each primer and 2.5 U Taq DNA polymerase (Promega, Madison, WI) within the standard reaction mix (3). Each cycle consisted of 1 min at 94°C, 1 min at 50°C and 1.5 min at 72°C generating a 973-bp fragment.

Primer Sequences:
5′ end 5′-GGAAAAACAATATGGCTACA-3′
3′ end 5′-TCTTCCTCTAGTCCTCACAA-3′

Polymorphism: TaqI detects a two-allele polymorphism at the CCAATT box (nucleotide −199) of the tyrosinase promoter region. The polymorphism consists of a single base substitution where the allele CCAATTCGA (D1) contains a TaqI site and the other allele, CCAATTAGA (D2), does not. Digestion of the 973-bp fragment (D2) results in two fragments, 199-bp and 774-bp versus a single fragment of 973-bp.

Frequency: Estimated from 28 unrelated normally pigmented unrelated Caucasian individuals.
D1 (199-bp fragment and 774-bp fragment): .893
D2 (973-bp fragment): .107
Observed heterozygosity = 21.4%
Unlike the polymorphism at codon 192 within exon I of the tyrosinase gene where all Orientals exhibited the same allele, both alleles were found in Oriental individuals (4, 5).
Estimated frequency from 40 normally pigmented unrelated Oriental individuals.
D1 (199-bp fragment and 774-bp fragment): .887
D2 (973-bp fragment): .113
Observed heterozygosity = 22.5%

Chromosomal Localization: 11q14 → 21 (6).

Mendelian Inheritance: Autosomal codominant inheritance was observed in two families.

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