Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2)

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Source/Description: The polymorphic (AAAG)_n repeat begins at base pair 176 of the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) on chromosome 6 (1). The polymorphism can be typed using the polymerase chain reaction (PCR) as described previously (2). The predicted length of the amplified sequence was 291 bp.

Primer Sequences:

AATCTGGGCGACAAGAGTGA (AAAG strand)
ACATCTCCCTACCGCTATA (TTTC strand)

Frequency: Estimated from 78 chromosomes of unrelated individuals. Heterozygosity Index = 93%. PIC = 0.93.

Allele (bp)	Frequency	Allele (bp)	Frequency
A1 318	0.01	A12 270	0.03
A2 314	0.04	A13 266	0.01
A3 310	0.05	A14 262	0.04
A4 306	0.10	A15 258	0.14
A5 302	0.09	A16 254	0.06
A6 298	0.09	A17 250	0.02
A7 294	0.03	A18 246	0.04
A8 290	0.04	A19 242	0.05
A9 282	0.03	A20 238	0.05
A10 278	0.03	A21 234	0.01
A11 274	0.04		

Mendelian Inheritance: Co-dominant segregation was observed in two informative families.

Chromosomal Localization: We have tentatively assigned the human beta-actin related pseudogene H-beta-Ac-psi-2 to chromosome 6 using rodent/human somatic cell hybrids.

Other Comments: The PCR reaction was performed on 80 ng of genomic DNA using 100 pmoles of each oligonucleotide primer. The samples were processed as described (3) except that the denaturation cycle at 94°C was extended to 1.4 minutes. The tetranucleotide repeat was based on a (AAAG)₁₁A₂(AAAG)₁₅ sequence.

References: 1) Moos, M. and Gallwitz, D. (1983) *EMBO J.* **2**, 757–761. 2) Weber, J.L. and May, P.E. (1989) *Am. J. Hum. Genet.* **44**, 388–396. 3) Weber, J.L. et al. (1990) *Nucleic Acids Res.* **18**, 4637.

A new G to T polymorphism in the retinoblastoma gene RB1 detected by DGGE

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Source/Description: Analysis by denaturing gradient gel electrophoresis (1) of PCR products, followed by direct sequencing revealed a G to T polymorphism in the intronic sequence downstream of exon 4. This polymorphism eliminates a cleavage site for MaeIII.

Primer Sequences:

RB7: 5'TGAGCTAACATTAAAAGGGA 3'
RB8: 5'AGTAGTGATTGTAGTAGAG 3'

Polymorphism: This new polymorphism occurs at 23 bp from the 3' end of exon 4. The polymorphic sequence is:

G
5' CATT TTTATTAGG TTACACTCTG 3'
T

Frequency: Estimated from 98 unrelated subjects.

Allele N1 (G): 94%

Allele N2 (T): 6%

Chromosomal Location: 13q14.

Mendelian Inheritance: Confirmed in 5 families.

Comments: For use in DGGE, the RB8 primer was GC-clamp extended at the 5' end (sequence of the clamp: GCCCGCCGCGCCCGACCCCGCGTCCGGCGCCCG).

Protocol: The two primers were used to amplify a 411bp long fragment containing exon 4 of the RB1 gene and intronic flanking sequences. 1μg of genomic DNA, 20 pM of each primer, 50 mM KCl, 10 mM Tris (pH 8.4), 2 mM MgCl₂, 200μM dNTP, 100 mg/mL gelatin, and 2.5U Taq polymerase (Cetus) were mixed in 100 μL volume. Amplification conditions consisted of 35 cycles of denaturing at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1.5 min. The PCR cycles were followed by a 10 minutes denaturing step at 94°C and a one hour reannealing step at 56°C. The products were analysed by denaturing gradient gel electrophoresis, and by direct sequencing of those that displayed altered electrophoretic behavior.

References: 1) Lerman, L. et al. (1987) *Methods Enzymol.* **155**, 501–527.