Dinucleotide repeat polymorphism at the D6S105 locus

J.L.Weber, A.E.Kwitek, P.E.May and H.Y.Zoghbi[1]
Marshfield Medical Research Foundation, 1000 North Oak Avenue, Marshfield, WI 54449 and [1]Baylor College of Medicine, Department of Pediatrics, One Baylor Plaza, Houston, TX 77030, USA

Source/Description: A human genomic *Sau3A* I fragment was cloned into mp19 and selected by hybridization to poly(dC-dA)·poly(dG-dT). The cloned fragment was designated Mfd61. Sequencing of Mfd61 provided the information necessary for polymerase chain reaction primer synthesis. The clone length was 214 bp, and the predicted length of the amplified fragment was 131 bp.

Primer Sequences: GCCCTATAAAATCCTAATTAAC (CA strand); GAAGGAGAATTGTAATTCCG (GT strand).

Frequency: Estimated from 62 chromosomes of unrelated CEPH family members (Caucasians). PIC = 0.77.

Allele (bp)	Frequency	Allele (bp)	Frequency
138	0.03	128	0.39
136	0.03	126	0.08
134	0.05	124	0.13
132	0.11	122	0.02
130	0.14	116	0.02

Chromosomal Localization: Assigned to chromosome 6p using DNA templates isolated from panels of somatic cell hybrids. Typing of CEPH families 1332 and 884 led to maximum LOD scores of 6.9 at $\theta = 0$ with HLA-B and 4.4 at $\theta = 0.07$ with HLA-DR. The use of a panel of 6p radiation hybrids gave the following order of markers: pter-D6S88-D6S108-D6S105-HLA-cen (1).

Mendelian Inheritance: Co-dominant segregation was observed in 6 multi-generation families.

Other Comments: Conditions for the amplification reactions were as described (2) except that samples were processed through 27 temperature cycles consisting of 1 min at 94°, 2 min at 55° and 1 min at 72°. Sizes of the alleles were determined by comparison to mp8 DNA sequencing ladders. The most intense band for each allele on the denaturing polyacrylamide gels was used to obtain allele size. The dinucleotide repeat sequence in Mfd61 was of the form $(CA)_{23}$. The sequence of Mfd61 has been submitted to GenBank.

Acknowledgements: This work was supported by the Marshfield Clinic and NIH grant HG00248.

References: 1) Zoghbi,H.Y., McCall,A.E. and LeBorgne-Demarquoy,F. (1990) *Am. J. Hum. Genet.* **47**, A206. 2) Weber,J.L. and May,P.E. (1989) *Am. J. Hum. Genet.* **44**, 388–396.

RsaI polymorphism in c-Ki-ras

J.Heighway
Department of Cancer Genetics, CRC Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, UK

Source/Description: Oligonucleotide primers were used to amplify a 1.36 kb region including c-Ki-ras exon 2 and sequence from the flanking 3′ intron (1).

PCR Primers: 528 5′ GGAAGCAAGTAGTAATTGATGGAG 3′
547 5′ TCATAATAGTAAAACAGAAACTACTG 3′

Polymorphism: RsaI identifies a simple two allele polymorphism.

Constant fragments 340 and 70 bp
A1 950 bp A2 680 and 270 bp

Frequency: Studied in 68 unrelated individuals
A1 0.77
A2 0.23.

Not Polymorphic For: No polymorphisms were detected in a panel of nine individuals using Sau96A, HinfI, HaeIII, TaqI, DdeI or HpaII.

Chromosomal Location: c-Ki-ras has been localised to 12p12.1 (2).

Mendelian Inheritance: Co-dominant segregation demonstrated in three families (14 individuals).

PCR Conditions: 1 μg of genomic DNA was amplified using 1 μg of each primer, 2 units of Taq polymerase (BCL) in 0.25 mM dNTPs and the reaction buffer provided (BCL). After heating at 97°C for 5 min, enzyme was added and the samples cycled 30 times at 94°C for 1 min, 55°C for 1 min and 74°C for 6 min.

Acknowledgements: This work was supported by the Cancer Research Campaign.

References: 1)McGrath *et al.* (1983) *Nature* **304**, 501–506. 2) HGM 10 (1989) *Cytogenetics and Cell Genetics* **51**, 1–1148.