PCR detection of a *Bgl*III RFLP at 11p13

Gail E.Tomlinson, Duane A.Compton¹, Louise C.Strong and Grady F.Saunders^{1*}

Department of Experimental Pediatrics and ¹Department of Biochemistry and Molecular Biology, The University of Texas M.D.Anderson Cancer Center, Houston, TX 77030, USA

Source/Location: Previously a *Bgl*III RFLP was described (1) using p8B1.25, a single copy probe (D11S325) located on 11p13 distal to the catalase locus and proximal to the Wilms tumor locus (2).

Polymorphism: The polymorphic *Bgl*III site lies within the probe approximately 400 nucleotides from the 5' end. Southern blotting of human genomic DNA detects two alleles: A1 = 16 kb, A2 = 11 + 5 kb (1). Amplification by pcr followed by *Bgl*III digestion identifies the same alleles as A1 = 750 bp, A2 = 550 bp + 200 bp.

Mendelian Inheritance: Codominant segregation as previously described and illustrated in the figure below.

PCR Primers: Forward: GACAGACACAGAGGAGAGAATGAATATAT
Reverse: CCAGTGCAGCAGAAGCAAAGCGCGG

PCR Conditions: PCR is carried out in a volume of 100 μ l containing 0.5 to 1.0 μ g genomic DNA, 25 pmoles of each primer, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris pH 8.2, 0.0001% gelatin, 200 μ M dNTP, 10% DMSO, and 3 units Taq DNA polymerase. Genomic DNA is denatured by heating to 95°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 1 min. The amplified product is then digested with *Bgl*III and fragments resolved by 2% agarose gel electrophoresis.

Comment: This method may be use to detect loss of heterozygosity in tumor specimens provided tumor is free of contaminating normal tissue.

Chromosomal Location: 11p13 by restriction mapping.

References: 1) Huff,V., Compton,D., Strong,L. and Saunders,G. (1990) *Human Genetics* **84**, 253–257. 2) Compton,D., Weil,M., Jones,C., Riccardi,V., Strong,L. and Saunders,G. (1988) *Cell* **55**, 827–836.

Dinucleotide repeat polymorphism at the human MHC class I CD8 alpha-chain (Leu-2/T8) gene

Mihael H.Polymeropoulos, Hong Xiao, Denise S.Rath and Carl R.Merril

National Institute of Mental Health Neuroscience Center, St. Elizabeths Hospital, Room 131, Martin Luther King Avenue, Washington, DC 20032, USA

Source/Description: The polymorphic (AC)_n repeat begins at base pair 3526 in intron E of the human MHC class I CD8 alpha-chain (Leu-2/T8) gene on chromosome 2p12 (1). The polymorphism can be typed using the polymerase chain reaction (PCR) as described previously (2). The predicted length of the amplified sequence was 145 bp.

Primer Sequences: ACTGCCTCATCCAGTTTCAG (AC strand);
GAGCAGGCACCTTGTTAGATG (GT strand).

Frequency: Estimated from 46 chromosomes of unrelated individuals. Observed heterozygosity = 71%. PIC = 0.66.

Allele (bp)	Frequency	Allele (bp)	Frequency
C1: 170	0.02	C5: 144	0.39
C2: 166	0.09	C6: 142	0.33
C3: 148	0.02	C7: 138	0.02
C4: 146	0.13		

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

Chromosomal Localization: The human MHC class I CD8 alpha-chain (Leu-2/T8) gene has been assigned to chromosome 2p12 (1).

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 dinucleotide repeat was based on a (AC)₁₄ sequence.

References: 1) Nakayama,K.I. *et al.* (1989) *Immunogenetics* **30**, 393–397. 2) Weber,J.L. and May,P.E. (1989) *Am. J. Hum. Genet.* **44**, 388–396. 3) Weber,J.L. *et al.* (1990) *Nucl. Acids Res.* **18**, 4637.

* To whom correspondence should be addressed