Primers for detection of *Stu*I and *Bst*XI polymorphisms in a fragment co-amplified with c-Ki-ras 2 (KRAS2)

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Source/Description: PCR primers for the amplification of a region of DNA found to be co-amplified with c-Ki-ras 2 in several human tumours. Southern analysis using the original fragment (1.3 kb *Hind*III/*Eco*RI, genomic) allowed detection of polymorphisms with *Stu*I and *Bst*XI (1) and the locus was shown to be closely linked to c-Ki-ras on chromosome 12 (2). Inverse PCR has been carried out in order to clone the flanking region that includes these sites. Sequence data was then used for synthesis of primers allowing both polymorphisms to be analysed in a single amplification reaction product.

PCR Primers:

255 5' GAGAAGGTATCCATGGGGAAGG 3'
510 5' CCAACAATAAAGGCTCTTTCAGAT 3'

Polymorphisms: The amplification product was 1200 bp.

<i>Stu</i> I		<i>Bst</i> XI	
B1	1200	constant	340
B2	950 and 250	F1	860
		F2	590 and 260

Frequency:

<i>Stu</i> I (35 individuals)	<i>Bst</i> XI (40 individuals)
B1 0.36 B2 0.64	F1 0.72 F2 0.28

Chromosome Location: 12p12.1 (2).

PCR Conditions: Amplifications were carried out on 1 µg of genomic DNA using 1 µg of each primer and 2.5 units of Taq polymerase (BCL) in the buffer provided. Reactions were heated at 97°C for 5 min before the enzyme was added and cycled 30 times at 94°C for 1 min, 61°C for 1 min and 74°C for 3 min.

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References: 1) Heighway, J. (1989) *Nucl. Acids Res.* **17**, 10515.
2) Hall *et al.* (1989) *Am. J. Hum. Genet.* **44**, 577–584.

*Stu*I and *Bst*XI digests of PCR generated products from six individuals. Marker track (centre) is a ϕ X174/HaeIII digest.

Variation within intron 3 of the apolipoprotein CII gene

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Source/Description: The PCR primers 5'-TAGATGAG-AACTC AGG-3' and 5'- GCTTTTGCTGTACAAGT-3' amplified apoCII intron 3 in a Tempcycler (Coy) using an annealing temperature of 60°C. Products were separated in 4% PAGE gels (Figure 1A).

Polymorphism: A two-allele polymorphism was detected, whose DNA sequence basis was a variation in the number of tandem repeats of a 37 base *tau*-like core sequence (1, 2): the 375 bp fragment corresponded to an allele with 7 repeat units (Allele K1) while the 335 bp fragment corresponded to an allele with 6 repeat units (Allele K2).

Frequency: Among 160 alleles studied to date, 18% were 375 bp (Allele K1), 82% were 335 bp (Allele K2).

Chromosomal Localization: Chromosome 19q13.3 (1).

Mendelian Inheritance: Co-dominant segregation was observed in three two generation families and one three generation family.

Other Comments: Dideoxy sequencing both of cloned PCR fragments and directly on PCR fragments revealed substantial sequence heterogeneity among the repeats at the same sequence position from many different alleles (Figure 1B).

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References: 1) Das *et al.* (1987) *J. Biol. Chem.* **262**, 4787–4793.
2) Rogaev (1989) *Nucl. Acids Res.* **17**, 1246.