Sspl polymorphism in sequence encoding 3' untranslated region of the APC gene

J.Heighway, P.R.Hoban and A.H.Wyllie¹

CRC Department of Cancer Genetics, Paterson Institute for Cancer Research, Wilmslow Road, Manchester, M20 9BX and ¹Cancer Research Campaign Laboratories, Department of Pathology, University of Edinburgh, Medical Building, Teviot Place, Edinburgh, EH8 9AG, UK

Source/Description: PCR primers for the amplification of an 850 bp fragment encoding part of the 3' untranslated region of the APC gene (DP2.5, ref. 1). Mutations within this gene are responsible for the Mendelian dominant disorder, familial polyposis coli.

Primers:

37A 5'GCATTAAGAGTAAAATTCCTCTTAC 3'

37B 5'ATGACCACCAGGTAGGTGTATT 3'

Polymorphism/Frequency: SspI identifies a simple two allele polymorphism:

	Digest products	Frequency
a1	270, 580 bp	0.46
a2	135, 135, 580 bp	0.54

Observed heterozygosity 21/40 unrelated individuals.

Chromosomal Localisation: 5q21.

Mendelian Inheritance: Co-dominant segregation demonstrated in three families (12 individuals).

PCR Conditions: 1 µg of genomic DNA was amplified in a 100 µl reaction mix containing dNTPs (0.25 mM), 0.5 µg of each oligo and Taq buffer (BCL). After heating for 2.5 min at 97°C, 2.5 units of Taq polymerase was added and reactions cycled at 94°C for 1 min, 55°C for 1 min and 74°C for 2.5 min (Grant Autogene II).

Comment: As the polymorphism is within a region of the gene encoded within the APC mRNA molecule it can be used to analyze both cDNA, in studying allele specific expression and chromosomal DNA, in establishing inheritance in families affected by familial polyposis (2). This polymorphism will also be of particular value in studying somatically acquired allele loss in 5q21 in sporadic colorectal carcinomas.

Acknowledgements: This work was supported by the Cancer Research Campaign and Royal Manchester Childrens Hospital (PRH).

References: 1) Joslyn *et al.* (1991) *Cell* **66**, 601–613. 2) Dunlop *et al.* (1991) *Lancet* **337**, 313–316.

Figure shows digested amplification products from five individuals. Marker track (left) is a ϕ X174 HaeIII digest.

PCR primers to convert c-Ki-ras to a four allele RsaI system (KRAS2)

J.Heighway

CRC Department of Cancer Genetics, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 9BX, UK

Source/Description: PCR primers to amplify a region of intron between c-Ki-ras2 exons 2 and 3. This fragment encodes a previously described polymorphic RsaI site (1). A simple polymorphism (C or A) was identified at position 19,744 of the genomic sequence (2). On amplification primer 771 created an RsaI site if the first base in the genomic sequence was a C. No site was formed if the base was an A.

PCR Primers: Mismatched base in lower case:-

771 5' TGCTAATGCCATGCATATAATATTTAgTA 3'

547 5' TCATAATAGTAAACAGAACTACTG 3'

Polymorphism: Use of primer 771 creates a system with two polymorphic RsaI sites. The original polymorphic fragments (A1, A2) are unchanged (1). The previously reported constant band of 340 bp is converted to a variant band:-
A3 190 bp A4 160 and (30) bp.

Frequency: Studied in 39 unrelated individuals

A3 0.71

A4 0.29

Observed heterozygosity = 44%

Chromosomal Localisation: c-Ki-ras (KRAS2) has been localised to 12p12.1.

Mendelian Inheritance: Co-dominant segregation of the second polymorphism has been demonstrated in three families (16 individuals).

PCR Conditions: 1 µg of genomic DNA was amplified in a 100 µl reaction mix containing dNTPs (0.25 mM), 1 µg of each oligo and Taq buffer (BCL). After heating for 4 min at 97°C, 2.5 units of Taq polymerase (BCL) was added and reactions cycled 30 times at 94°C for 1 min, 50°C for 1 min and 74°C for 3 min (Grant Autogene II).

Comment: The two polymorphic sites are independent of each other (observed heterozygosity of 59% in 39 individuals; predicted heterozygosity if independent, 62%).

Acknowledgement: This work was funded by the Cancer Research Campaign.

References: 1) Heighway, J. (1991) *Nucl. Acids Res.* **19**, 968. 2) McGrath *et al.* (1983) *Nature* **304**, 501–506.

Figure shows amplification products digested with RsaI.