Polymorphism in exon 1 of the c-Ha-ras gene, (HRAS)

P.R.Hoban, M.F.Santibanez-Koref and A.M.Kelsey¹
CRC Department of Cancer Genetics, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX and ¹Department of Pathology, Royal Manchester Childrens Hospital, Pendlebury, Manchester M27 1HA, UK

Source/Description: At codon 27 of the c-Ha-ras oncogene there is a single base pair polymorphism previously described as a sequencing variant (1). We have designed an oligonucleotide primer (P3) which is able to detect the polymorphism through incorporation of the polymorphic base into an *Nsi*I restriction site. P3 contains 2 mismatches (underlined) at the 3' end of the primer which is immediately adjacent to the polymorphism. Two step amplification using P1 and P2 followed by amplification with P2 and P3 results in a 112 bp fragment.

PCR Primers:

P1: 5'TCAAGCTTGGCAGGTGGGGCAGGAG 3'
P2: 5'TAGGATCCTGGGCTCGCCCGCAGCA 3'
P3: 5'TGACCATCCAGCTGATCCAGATGCA 3'

Method: 1 µg of genomic DNA was amplified using 0.1 µM of P1 and P2 in a total volume of 100 µl containing 1×*Taq* polymerase buffer (BCL) and 200 µM dNTPs. Samples were heated to 97°C for 5 min after which 2 units of *Taq* polymerase were added. Reactions were cycled 15 times at 92°C for 1 min, 60°C for 45 seconds and 74°C for 1 min. Following this 2 µl aliquots were transferred to tubes containing 98 µl PCR reaction mixture containing 0.5 µM P2 and P3. Samples were treated as above, following the addition of *Taq* polymerase (2 units) reactions were cycled 25 times at 92°C for 1 min, 55°C for 1 min and 74°C for 1 min. Amplified products and restriction enzyme digests (10 µl of amplified product in a total volume of 50 µl) were analyzed on 3% agarose gels.

Polymorphism: Digestion with *Nsi*I reveals a two allele system, allele 1 (J1) is 112 bp and allele 2 (J2) is 87 bp and 25 bp.

Frequency: Determined from a population of 60 unrelated individuals J1: 0.33; J2: 0.66.

The observed heterozygote frequency is 46%.

Mendelian Inheritance: Co-dominant segregation was observed in six families (34 individuals).

Chromosomal Localisation: c-Ha-ras has been localised to 11p15.5.

Comment: As this polymorphism is located within a transcribed region of the Ha-ras gene, using cDNA specific primers it is possible to examine allele specific expression on RNA samples from heterozygote individuals (see figure).

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Reference: 1) Taparowsky, E. *et al.* (1982) *Nature* **300**, 762–765.

HindIII RFLP for NRAS in *Monodelphis domestica*

R.van Oorschot, V.Birmingham and J.L.VandeBerg*
Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78228, USA

Source and Description of Clone: p52 c⁻, a 1.5 kb fragment encoding 3' untranslated region of human NRAS [neuroblastoma RAS viral (v-ras) oncogene homolog], provided to the ATCC by R.A.Weinberg (Murray *et al.* 1983).

Polymorphism: HindIII identifies a 2-allele polymorphism with a band at either 11.5 kb (A1), or 8.5 kb (A2).

Frequency: Among 71 *M.domestica* animals, allele A1 (11.5 kb) 0.32 and allele A2 (8.5 kb) 0.68. Twenty-five percent were heterozygous.

Not Polymorphic For: EcoRI, BglII or BamHI with DNA from 16 animals.

Chromosomal Location: Unknown in *M.domestica*, human chromosome 1p13 (Human Gene Mapping, 1989).

Mendelian Inheritance: Co-dominant segregation was demonstrated in 5 *M.domestica* families.

Probe Availability: ATCC #41030.

Other Comments: *Taq*I and *Pst*I also show polymorphism in *M.domestica*.

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References: 1) Murray *et al.* (1983) *Cell* **33**, 749–757. 2) Human Gene Mapping (1989) *Cytogenet. Cell Genet.* **51**, 1–1148.

* To whom correspondence should be addressed