A TaqI RFLP in the region of the HPRT locus

P.Renwick, M.Raybould, A.Birley¹ and M.Hultén
DNA Laboratory, Regional Cytogenetics, East Birmingham Hospital, Birmingham B9 5PX and ¹Population and Environmental Research Group, School of Biological Sciences, The University of Birmingham, B15 2TT, UK

Source/Description: EcoRI restriction fragments (total length 18.5 kb) were isolated from the bacteriophage Huλ2 to provide material for a human genomic DNA probe, MAJ1001. Huλ2 contains genomic DNA sequences derived from the *hprt* region (X-chromosome) that are cloned into the EcoRI target sites of the bacteriophage Charon 4A (1). The probe sequence originates from exon 7 and extends 14.4 kb into the *hprt* 3' flanking region.

Polymorphism: This probe revealed a two allele restriction fragment length polymorphism with bands at 1.8 kb and 2.2 kb (A1) or 4.0 kb (A2) after Southern blot analysis of human genomic DNA digested with TaqI.

Frequency: Estimated from 30 unrelated British individuals (44 X-chromosome total). A1 (1.8 + 2.2 kb) = 0.89; A2 (4.0 kb) = 0.11. Of 14 females tested, 4 were heterozygous. No homozygous females were observed for allele A2. Constant bands of 0.4, 0.5, 0.8, 1.0, 1.6 and 3.4 kb.

Chromosomal Location: The *hprt* gene has been localised to Xq26–27 (2).

Mendelian Inheritance: Codominant inheritance demonstrated in one family EBH1.

Other Comments: (a) The father of family EBH1 also has the 22-kb/18-kb allele for the previously described BamHI restriction fragment length polymorphism (3). (b) The presence of repetitive sequences in the probe was overcome by using 'Hybridime' genomic DNA blocking agent in the hybridisations (supplied by Stratech Scientific Limited). (c) Computer studies (4) of the *hprt* sequence (5) show that the TaqI RFLP is most likely caused by a single nucleotide change in the restriction site beginning at position 50988 which occurs within ALU number 44.

Acknowledgements: Huλ2 was a gift from P.I.Patel, Howard Hughes Medical Institute Laboratory, Baylor College of Medicine, Houston, TX 77030. The work is supported by the Letten F.Saugstads fund.

References: 1) Patel *et al.* (1986) *Mol. Cell. Biol.* **6**, 393–403. 2) Pai *et al.* (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2810–2813. 3) Nussbaum *et al.* (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4035–4039. 4) Devereux *et al.* (1984) *Nucl. Acids Res.* **12**, 387–395. 5) Edwards *et al.* (1990) *Genomics* **6**, 593–608.

Autoradiographs from the left: male hemizygous for allele A1, male hemizygous for allele A2, female heterozygous for alleles A1 and A2, female homozygous for allele A1, heterozygous female for alleles A1 and A2.

PstI polymorphism within the 3' untranslated region of the insulin gene detectable by the polymerase chain reaction (INS)

Paul R.Hoban and Anna M.Kelsey
Department of Pathology, Royal Manchester Childrens Hospital (RMCH), Pendlebury, Manchester M27 1HA, UK

Source/Description: Oligonucleotide primers to the 3' untranslated region of the insulin gene were used to amplify a 221 bp fragment. A sequence variant from within this region has previously been described (1).

PCR primers: 5' AAGCGTGGCATTGTGGAACAAT 3'
5' CTGGGAGGGGCTCACAAACAGT 3'

Method: 1 µg of genomic DNA was amplified using 0.5 µg of each primer in total volume of 100 µg 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 (BCL) were added. Reactions were cycled 30 times at 94°C for 1 min, 60°C for 1 min and 73°C for 2 mins. Restricted PCR products (10 µl aliquots digested in a total volume of 50 µl) were analyzed by electrophoresis through 3% agarose gels.

Polymorphism: PstI restriction reveals a two allele system, allele 1 is 221 bp and allele 2 is 137 bp and 84 bp.

Frequency: Determined from 70 unrelated individuals.

Allele 1 (A1): 0.71

Allele 2 (A2): 0.29

Heterozygosity is 0.30 (21/70 individuals studied).

Mendelian Inheritance: Codominant segregation was observed in three families (14 individuals).

Comment: As this polymorphism is located within a transcribed region of the insulin gene it should allow the PCR based determination of allele specific expression within RNA samples, in addition to the detection of allele imbalance within DNA samples.

Chromosomal Localisation: The insulin gene has been localised to 11p15.5 (2).

Acknowledgement: This work was supported by RMCH from the Pendlebury Leukaemia Research Fund.

References: 1) Ulrich, A. *et al.* (1980) *Science* **209**, 612–615. 2) Bell, G.I. (1982) *Nature* **295**, 31–35.