DGGE detection of HhaI polymorphism in the F9 gene

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Carrier detection of hemophilia B using RFLPs as linkage markers with F9 gene allowed us to diagnose about 69% of females at risk (1).

HhaI polymorphism detected with enzyme digestion on PCR product increases the percentage of informativity to 89% (2). To overcome the difficulty of enzyme digestion we tested this polymorphism with DGGE. With the synthesis of a 40 mer GC-clamp (3) to 5' end of the oligonucleotide used to amplify the DNA fragment by PCR the sensitivity of this technique is increased. The reaction is performed by a DNA Thermocycler (Perkin-Elmer-Cetus) according to the indication of Winship (2).

The 270 bp (230 + 40) fragments obtained without aspecific DNA, are loaded onto a 16×18 cm, 0.75 mm thick 6.5% polyacrylamide gel containing a 20–80% denaturant gradient parallel to the direction of electrophoresis (100% denaturant = 5 M urea 32% formamide). The gel runs at 80 volts for 13 h submerged in circulating TAE buffer at a constant temperature of about 65°C.

DNA fragments migrate to different positions in the gel according to their base composition and melting behaviour. The data obtained were compared with the enzymatic digestion: the allele (–) migrates slower than allele (+). The heterozygotes present two distinct bands.

In a sample of 65 chromosomes from unrelated Italian individuals the allele (+) has a frequency of 66% and the allele (–) of 34%.

We tested 47 DNA samples (35 females and 12 males) with HhaI polymorphism on DGGE. The figure shows a family study.

The identification of HhaI polymorphism with DGGE seems to be more effective than the enzymatic digestion on PCR products where we obtained several partial digestions.

References: Mori, P.G., Pecorara, M., Acquila, M., Caprino, D. and Panarello, C. (1988) *Haemophilia B Carrier Detection by Intragenic RFLP*. XVIII International Congress of the World Federation of Hemophilia. Madrid, April 26–31, 1988. p. 8, No. 183. 2) Winship, P.R., Rees, D.J.G. and Alkan, M. (1988) *Lancet* i, 631–634. 3) Cheffield, V.C., Cox, D.R., Lerman, L.S. and Myers, R.M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 232–236.

Figure 1. In this family the disease segregates with the allele + coming from the woman I.1. The female III.1 is not carrier.

Three polymorphisms at the D10S85 locus

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Source/Description: p4dIII/RI 0.55 is a 550 bp unique EcoRI/HindIII fragment in pUC8. It was subcloned from phage 4 isolated from a library in λ DASH made with DNA from the somatic cell hybrid pp10A (Goodfellow *et al.*, 1989; Goodfellow *et al.*, 1990).

Polymorphisms: MspI identifies a 3 allele polymorphism (A1 = 1.4 kb, A2 = 0.8 + 0.6 kb, A3 = 1.75 kb). BglII and TaqI each identify a 2 allele polymorphism (B1 = 6.8 kb, B2 = 19 kb and C1 = 1.8 kb, C2 = 1.1 kb respectively).

Allele Frequencies:

A1 0.61
A2 0.33
A3 0.06 80 chromosomes
B1 0.71
B2 0.29 113 chromosomes
C1 0.94
C2 0.06 120 chromosomes

Not Polymorphic For: RsaI, HindIII, PvuII, PstI and EcoRI.

Mendelian Inheritance: Co-dominant inheritance observed in several *MEN2A* and reference kindreds.

Chromosomal Localization: Localized to 10q22-q23 using somatic cell hybrids. Linkage analyses (tight linkage with D10S4) confirm localization.

Probe Availability: Contact P.J.G.

Other Comments: Normal hybridization/washing conditions.

References: 1) Goodfellow, P.J., Povey, S., Nevanlinna, H.A. and Goodfellow, P.N. (1989) *Human Gene Mapping 10*, Tenth International Workshop on Human Gene Mapping. *Cytogenet. Cell Genet.* **51**, 1007. 2) Goodfellow, P.J., Povey, S., Nevanlinna, H.A. and Goodfellow, P.N. (1990) *Somatic Cell Mol. Genet.* in press.