

## RsaI RFLP for electron transport flavoprotein-beta(ETFB)

V.Royal, M.J.Alberts\*, M.A.Pericak-Vance, G.Finocchiaro<sup>1</sup>, J.Bebout, L.Yamaoka, W.-Y.Hung, P.C.Gaskell and A.D.Roses

The Joseph and Kathleen Bryan Alzheimer's Disease Research Center and the Division of Neurology, Department of Medicine, Duke University Medical Center, Durham, NC 27710 and <sup>1</sup>The Department of Biochemistry and Genetics, Istituto Nazionale Neurologico, Celoria 11, 20133 Milan, Italy

**Source/Description:** The ETFB probe used was a 850 bp cDNA in the EcoRI site of plasmid pGEM7zf. The probe was supplied by Gaetano Finocchiaro.

**Polymorphism:** A two allele RFLP was detected using RsaI. Band sizes produced were 2.75 kb (A1) and 2.6 kb (A2)

**Frequency:** Analysis of 38 unrelated individual from 27 different families showed these frequencies:

A1 = 0.51

A2 = 0.49

**Other Enzymes:** Enzymes that also demonstrated polymorphisms with random placental DNA blots were: BamHI, BanI, BglII, HincII, NcoI, PstI, PvuII and StuI. These polymorphisms were not used because they were either less polymorphic than RsaI or because the DNA fragments were too small to detect reliably. Enzymes that did not demonstrate a polymorphism were: BglII, EcoRI, HindIII, HinfI, MspI, Xba, XmnI.

**Chromosomal Location:** ETFB has been mapped to Chromosome 19 using a panel of somatic cell hybrids (1).

**Mendelian Inheritance:** Co-dominant inheritance was demonstrated based on genotypes of 258 individuals from 27 families.

**Probe Availability:** Contact Dr. Finocchiaro at the Department of Biochemistry and Genetics, Istituto Nazionale Neurologico, Celoria 11, 20133 Milano Italy.

**Acknowledgements:** The authors wish to thank Dr. Finocchiaro for supplying the ETFB probe. This work was supported in part by grants AG05128 and AG07922 from the National Institute of Aging, and grant NS01241 from the National Institute of Neurologic Disorders and Stroke (NINDS).

**Reference:** 1) Williamson,R., Bowcock,A., Kidd,K., Pearson,P., Schmidtke,J., Chan,H.S., Chipperfield,M., Cooper,D.N., Hewitt,J., Lewitter,F., Maidak,B., Quitt,M., Ricciuti,F. and Track,R. (1990) Report of the DNA committee and catalogues of cloned and mapped genes and DNA polymorphisms. *Cytogenet. Cell Genet.* **55**, 457-778.

## Acyl-RFLP identified on an amplified region of the TPO gene

G.Rose<sup>1</sup>, C.Giacchetto<sup>2</sup> and G.De Benedictis<sup>1</sup>

<sup>1</sup>Cell Biology Department, University of Calabria, Rende (CS), and <sup>2</sup>Unità Endocrinologica, USSL n. 11 Calabria, Amantea (CS), Italy

Thyroid Peroxidase (TPO) is a thyroid-specific enzyme that plays a central role in thyroid hormone synthesis, catalyzing multiple steps in the biosynthetic pathway (1). Two primers (TPO-10a and TPO-10b) were used to amplify a 739 bp long fragment including exon 10 and parts of introns 9 and 10 of the human TPO gene (232 bp and 338 bp upstream and downstream exon 10 respectively) (2).

**Primers:**

TPO-10a 5'TAGCCAAGCATGGTAGCGGG 3'

TPO-10b 5'TAGCCATCCCAGGAGCTTCC 3'

After PCR amplification (30 cycles: 1' at 94°C, 30' at 64°C, 40' at 72°C) the amplified products were digested by AcyI restriction endonuclease. The one AcyI site reported in the published sequence at 1815 bp in exon 10 (2) should split the amplified product into two fragments of 359 bp and 380 bp. While the 380 bp was present in all the samples, the 359 bp band resulted clearly polymorphic with a further band of 340 bp (Fig. 1). An inspection of the reference sequence (2) showed that A→C mutation 212 bp upstream exon 10 originates the AcyI restriction site (Allele - : GGAGCC→Allele + : GGCGCC).

**Frequencies:** Estimated in 64 unrelated Caucasians

Allele + : 0.66;

Allele - : 0.34

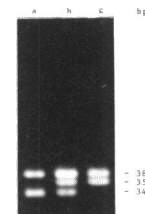
**Chromosomal Localization:** 2pter-p12 (3).

**Mendelian Inheritance:** Codominant segregation shown in 6 families (18 meioses).

**Comments:** Not polymorphic for: HinfI; PstI; AvaII in the same sample group.

**Acknowledgements:** Work supported by the Italian Ministry of University and Scientific Research (MURST) and by the Unità Sanitaria Locale (USSL) n. 11 Calabria (Italy).

**References:** 1) Nunez,J. (1980) *The Thyroid Gland* (De Visscher M. ed.) pp 39-59 Raven, New York. 2) Kimura,S. *et al.* (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5555-5559. 3) de Vijlder *et al.* (1988) *Cytogen. Cell Genet.* **47**, 170-172.



**Figure 1.** AcyI-RFLP of the hTPO amplified region.  
a : +/+; b : +/-; c : -/-

\* To whom correspondence should be addressed