PCR detection of the pKM.19/ScrfI RFLP (D7S23), a marker closely linked to the cystic fibrosis mutation

V.Nunes*, M.Chillón, N.Lench[1], M.Ramsay[1] and X.Estivill
Molecular Genetics Department, Fundació d'Investigació, Sant Pau, 08025 Barcelona, Spain and [1]Molecular Genetics Department, St Mary's Hospital Medical School, London W2 1PG, UK

Source/Description: pKM-19 is a 1.0 kb EcoRI human genomic fragment inserted in pUC13, that detects a ScrfI (CC/NGG) RFLP (1, 2). We report here the primer sequences suitable for the detection of this RFLP by PCR.

Polymorphism: ScrfI digest of the amplified fragments identifies two alleles: 412 bp (A1) and 212 + 200 bp (A2). Allele 1 represents the absence of the ScrfI site, and allele 2 the presence of the site (Figure).

Frequency: European Caucasoid Population from 320 unrelated individuals: CF (A1) = 0.67, (A2) = 0.33; non-CF (A1) = 0.04, (A2) = 0.96.

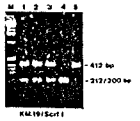
Chromosomal Localization: The polymorphic ScrfI recognition site maps approximately at 200 kb from the CFTR gene on 7q31 (ref. 3).

Mendelian Inheritance: Co-dominant segregation demonstrated in 98 families.

Other Comments: The oligonucleotide sequences for PCR are: MIV1: 5'CCTTCTAGGCTGTGTGGCT3' and VIM2: 5'GTGGCTCAGAGATTCTGCC3'. We carry out PCR as recommended by the supplier, using 400 ng of genomic DNA, 90 pmoles of each primer, 200 μM dNTPs, and 2 units of Taq polymerase (Cetus/Perkin Elmer). Initial denaturation for 5 min followed by 35 cycles of 15 sec 95°C, 45 sec 60°C, and 30 sec 74°C. The amplified products were digested overnight with ScrfI. DNA fragments are resolved by electrophoresis through a 2% agarose gel.

Acknowledgement: This work was supported by the Spanish Ministry of Education and Science (PB87–0074).

References: 1) Estivill *et al.* (1987) *Genomics* **1**, 257–263. 2) Nunes *et al.* (1990) *Nucl. Acids Res.* **18**, 1318. 3) Kerem,B.-S. *et al.* (1989) *Science* **245**, 1073–1080.



* To whom correspondence should be addressed

Tetranucleotide repeat polymorphism in the vWF gene

Hans K.Ploos van Amstel and Pieter H.Reitsma
Haemostasis and Thrombosis Research Unit, University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands

Source/Description: Two primers (vWFII-5 TGTACCTAGTTATCTATCCTG and vWFII-3 GTGATGATGATGGAGACAGAG) were used to amplify a 166 bp long TCTA repeat rich region (nt 2215–2380) of intron 40 of the human vWF gene (1).

Polymorphism: The PCR dependent DNA amplification with vWFII-5 and vWFII-3 detects six alleles designated WBII1–6 in decreasing order of size (see figure) 174, 170, 166, 162, 158 and 154 bp respectively.

Frequency: A1, 0.08; A2, 0.06; A3, 0.27; A4, 0.35; A5, 0.05; A6, 0.19 as estimated from 24 unrelated caucasians.

Chromosomal Localization: The vWF gene has been localized on chromosome 12 (2).

Mendelian Inheritance: Co-dominant inheritance.

Availability: Contact P.H.Reitsma.

Comments: The WBII alleles are not in linkage disequilibrium with the alleles found for the TCTA repeat rich region located upstream (nt 1900–2000) in the vWF gene (3). The conditions of the polymerase chain reactions were essentially as described (4) consisting of 35 cycles with denaturing at 94°C for 1 min annealing at 55°C for 1.5 min and extension at 71°C for 3 min. The fragments were visualized on a 0.4 mm thick 6% PAGE-gel by staining with ethidium bromide.

Acknowledgement: This work was supported by the Praeventiefonds (grant no. 28–1862).

References: 1) Mancuso,D.J.*et al.* (1989) *J. Biol. Chem.* **264**, 19514–19527. 2) Ginsburg,D. *et al.* (1985) *Science* **228**, 1401–1426. 3) Peake I.R. *et al.* (1989) *Thromb. Haemostas.* **62**, 472. 4) Kogan,S.C. *et al.* (1987) *N. Engl. J. Med.* **317**, 985–990.